CHAPTER IV

RESULTS

4.1 Characteristics of the Subjects

There were 41 subjects enrolled in this study (17 males and 24 females). The subjects were randomly assigned into two groups, the WPI or control groups. The WPI group comprised of 21 subjects (8 males and 13 females) who received 30 g of WPI daily for 6 weeks while the control group comprised of 20 subjects (9 males and 11 females). Five subjects did not complete the study because 2 subjects was lost to follow-up (1 in the WPI group and 1 in the control group), 1 subject (in the control group) had inflammation, and 2 subjects (in the WPI group) consumed WPI less than 80%. Therefore, there were totally 36 subjects participating throughout the study [14 males (38.9%) and 22 females (61.1%)]. The characteristics of the subjects are shown in **Table 7**.

The subjects who allocated to the WPI group were not statistically different from the control group with regard to sex, age, diabetes duration, existing disease, metabolic syndrome, and medication use. The WPI group included 18 subjects (6 males and 12 females), and the control group included 18 subjects (8 males and 10 females). Mean age of the WPI and control groups were 62.56 ± 2.46 and 59.72 ± 2.37 years respectively. Mean diabetes duration of the WPI and control groups were 5.01 ± 0.86 and 7.04 ± 1.76 years respectively. Fifty percent of the subjects had type 2 DM with hypertension and dyslipidemia. Most of the subjects had metabolic syndrome (94.4%). Dietary pattern and physical activity in the WPI and control groups were not significantly different (**Appendix F**).

Table 7 Characteristics of the subjects

Characteristics	WPI group (n=18)	Control group (n=18)	Total
	number (%)	number (%)	number (%)
Sex			
Males	6 (33.3)	8 (44.4)	14 (38.9)
Females	12 (66.7)	10 (55.6)	22 (61.1)
	$\chi^2 = 0.468$	df = 1	$p = 0.494$
Age (years)			
35-44	1 (5.6)	1 (5.6)	2 (5.6)
45-54	3 (16.7)	5 (27.7)	8 (22.2)
55-64	6 (33.3)	3 (16.7)	9 (25.0)
≥ 65	8 (44.4)	9 (50.0)	17 (47.2)
Mean of age[1] (years)	62.6 ± 2.5	59.7 ± 2.4	61.1 ± 1.7
	$\chi^2 = 1.559$	df = 3	$p = 0.669$
Diabetes duration (years)			
< 5	9 (50.0)	9 (50.0)	18 (50.0)
5- < 10	2 (11.1)	6 (33.3)	8 (22.2)
> 10	7 (38.9)	3 (16.7)	10 (27.8)
Mean of duration[1] (years)	5.0 ± 0.9	7.0 ± 1.8	6.1 ± 1.0
	$\chi^2 = 3.600$	df = 2	$p = 0.165$
Existing disease			
Type 2 DM	4 (22.2)	1 (5.6)	5 (13.9)
Type 2 DM+HT	5 (27.8)	2 (11.1)	7 (19.4)
Type 2 DM+dyslipidemia	3 (16.7)	3 (16.7)	6 (16.7)
Type 2 DM+HT+dyslipidemia	6 (33.3)	12 (66.7)	18 (50.0)
	$\chi^2 = 5.086$	df = 3	$p = 0.164$

[1]mean ± SEM

df = degree of freedom; χ^2 = compare frequency between groups by Chi-square test; WPI = whey protein isolate; DM = diabetes mellitus; HT = hypertension

Table 7 Characteristics of the subjects (continued)

Characteristics	WPI group (n=18)	Control group (n=18)	Total
	number (%)	number (%)	number (%)
Metabolic syndrome			
Yes	16 (88.9)	18 (100.0)	34 (94.4)
No	2 (11.1)	0 (0.0)	2 (5.6)
	$\chi^2 = 2.118$	df = 1	$p = 0.146$
Medication usage			
Oral hypoglycemic agents			
Sulfonylureas	3 (16.7)	1 (5.6)	4 (11.1)
Bigaunides	3 (16.7)	0 (0.0)	3 (8.3)
Sulfonylureas + Bigaunide	12 (66.7)	17 (94.4)	29 (80.6)
	$\chi^2 = 4.862$	df = 2	$p = 0.088$
Antihypertensive agents[1] or Antidyslipidemic agents			
No	4 (22.2)	1 (5.6)	5 (13.9)
Yes	14 (77.8)	17 (94.4)	31 (86.1)
Antihypertensive agents	4 (22.2)	2 (11.1)	6 (16.7)
Antidyslipidemic agents	3 (16.7)	3 (16.7)	6 (16.7)
Antihypertensive agents +Antidyslipidemic agents	7 (38.9)	12 (66.7)	19 (52.8)
	$\chi^2 = 2.090$	df = 1	$p = 0.148$

[1]Antihypertensives agents = Moduretic, Beta blockers, Calcium blockers, or Angiotensin converting enzyme inhibitors; WPI = whey protein isolate

4.2 Baseline Clinical Parameters of the Subjects

The baseline clinical parameters of the subjects in the WPI and control groups are summarized in **Table 8**. None of the measured parameters differed between groups ($p > 0.05$). The mean hs-CRP level at baseline of the subjects in the WPI and control groups in this study were 2.32 ± 1.01 mg/l and 2.48 ± 0.50 mg/l respectively. The mean plasma IL-6 level of the subjects in the WPI and control groups at baseline were 1.95 ± 0.25 pg/ml and 1.92 ± 0.17 pg/ml respectively.

Table 8 Baseline clinical parameters of the subjects[1]

Clinical parameters	WPI group (n = 18)	Control group (n = 18)
Body weight (kg)	63.24 ± 1.92	63.11 ± 2.25
BMI (kg/m^2)	25.92 ± 0.60	25.18 ± 0.59
SBP (mmHg)	127.50 ± 3.19	123.67 ± 3.97
DBP (mmHg)	73.83 ± 1.84	75.56 ± 2.46
FBS (mg/dl)	123.39 ± 5.64	114.39 ± 5.51
HbA1c (%)	6.88 ± 0.21	7.26 ± 0.37
TC (mg/dl)	187.78 ± 6.67	199.28 ± 6.05
TG (mg/dl)	141.89 ± 8.97	140.72 ± 10.86
Albumin (g/dl)	4.58 ± 0.07	4.56 ± 0.05
hs-CRP (mg/l)	2.32 ± 1.01	2.48 ± 0.50
IL-6 (pg/ml)	1.95 ± 0.25	1.92 ± 0.17

[1]mean ± SEM.
WPI = whey protein isolate; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; FBS = fasting blood sugar; HbA1c = hemoglobin A1c; TC = total cholesterol; TG = triglyceride; hs-CRP = high sensitivity C-reactive protein; IL-6 = interleukin-6; kg/m^2 = kilogram per metre square; mmHg = millimetre of mercury; mg/dl = milligram/deciliter; mg/l = milligram/liter; pg/ml = picogram/milliliter

Correlations of IL-6, hs-CRP and other variables in the control and WPI groups at baseline are shown in **Table 9**. The data showed significant correlations between hs-CRP and IL-6 (r = 0.450, $p < 0.01$), SBP and DBP (r = 0.728, $p < 0.01$), SBP and weight (r = 0.371, $p < 0.05$), SBP and BMI (r = 0.408, $p < 0.05$), DBP and weight (r = 0.508, $p < 0.01$), DBP and BMI (r = 0.384, $p < 0.05$), FBS and HbA1c (r = 0.453, $p < 0.01$), TC and BMI (r = 0.347, $p < 0.05$), weight and BMI (r = 0.661, $p < 0.01$).

Table 9 Partial correlation analysis of inflammatory mediators with variables of the subjects at baseline

Variables	hs-CRP	IL-6	SBP	DBP	FBS	HbA1c	TC	TG	Weight	BMI
hs-CRP										
correlation	1	0.450**	0.093	0.018	-0.001	0.175	0.114	-0.110	0.200	0.235
IL-6										
correlation	0.450**	1	-0.058	-0.298	-0.035	-0.030	-0.290	-0.231	-0.173	-0.013
SBP										
correlation	0.093	-0.058	1	0.728**	0.119	-0.092	0.308	-0.232	0.371*	0.408*
DBP										
correlation	0.018	-0.298	0.728**	1	0.125	-0.072	0.332	-0.036	0.508**	0.384*
FBS										
correlation	-0.001	-0.035	0.119	0.125	1	0.453**	0.251	0.084	0.246	0.190
HbA1c										
correlation	0.175	-0.030	-0.092	-0.072	0.453**	1	0.146	0.221	0.114	-0.054
TC										
correlation	0.114	-0.290	0.308	0.332	0.251	0.146	1	0.198	0.251	0.347*
TG										
correlation	-0.110	-0.231	-0.232	-0.036	0.084	0.221	0.198	1	0.020	0.299
Weight										
correlation	0.200	-0.173	0.371*	0.508**	0.246	0.114	0.251	0.020	1	0.661**
BMI										
correlation	0.235	-0.013	0.408*	0.384*	0.190	-0.054	0.347*	0.299	0.661**	1

hs-CRP = high sensitivity C-reactive protein; IL-6 = interleukin-6; SBP = systolic blood pressure; DBP = diastolic blood pressure; FBS = fasting blood sugar; HbA1c = Hemoglobin A1c;TC = total cholesterol; TG = triglyceride; BMI = body mass index
*Significant correlation ($p < 0.05$) **Significant correlation ($p < 0.01$)

4.3 Dietary Intake of the Subjects

The subjects were advised about proper food intake. Energy distribution and nutrient intakes were assessed by 3-day food records at the beginning (baseline) and at the end of the experimental period (week 6) in both groups. All records were analyzed in terms of total energy, total protein, protein from animal, protein from vegetable, fat, cholesterol, carbohydrate, sugar, fiber, and water intakes per day. (**Table 10**)

The result showed that energy distribution and nutrient intakes of the subjects in the WPI and control groups at baseline were not significantly different. In the WPI group, the amount of total protein and protein from animal consumption at week 6 of the experimental period were significantly greater than those at baseline. It was found that the amount of carbohydrate and protein from vegetable consumption at week 6 of the experimental period in the WPI group were significantly less than those at baseline. At week 6 after WPI supplementation the amount of total protein and protein from animal intakes in the WPI group were significantly greater than those in the control group, but the amount of carbohydrate and protein from vegetable intakes in the WPI group were significantly less than those in the control group ($p < 0.001$). At week 6 of the experimental period, the increase in amount of carbohydrate consumption was observed in the control group from 191.05 ± 6.93 g/day at baseline to 201.23 ± 8.53 g/day ($p = 0.019$).

Table 10 Dietary intake of the subjects at baseline and week 6 of the experimental period [1]

Dietary intake	WPI group		Control group	
	Baseline	**Week 6**[2]	**Baseline**	**Week 6**
Total energy				
kcal/day	1406.45 ± 46.02	1349.03 ± 47.56	1400.58 ± 42.70	1414.54 ± 49.61
Total protein				
kcal/day	255.16 ± 19.32	326.36 ± 17.16*[††]	249.24 ± 14.68	243.08 ± 10.28
g/day	63.79 ± 4.83	81.59 ± 4.29*[††]	62.31 ± 3.67	60.77 ± 2.57
g/kg/day	1.03 ± 0.08	1.32 ± 0.08*[†]	1.00 ± 0.06	0.98 ± 0.05
% of total energy	17.93 ± 0.99	24.27 ± 0.98*[††]	17.50 ± 1.10	16.86 ± 0.45
Protein from animal				
g/day	40.13 ± 4.93	61.30 ± 4.66*[††]	36.73 ± 4.36	32.74 ± 1.96
Protein from vegetable				
g/day	16.90 ± 0.79	12.27 ± 0.72*[†]	16.35 ± 1.09	16.64 ± 1.46
Fat				
kcal/day	321.12 ± 25.38	337.14 ± 25.02	403.47 ± 24.75	356.13 ± 27.09
g/day	35.68 ± 2.82	37.46 ± 2.78	44.83 ± 2.75	39.57 ± 3.01
% of total energy	22.75 ± 1.67	24.61 ± 1.24	28.81 ± 1.54	24.75 ± 1.50
Cholesterol				
kcal/day	1.63 ± 0.21	1.60 ± 0.27	1.82 ± 0.17	1.62 ± 0.16
mg/day	181.15 ± 23.37	177.32 ± 30.14	202.09 ± 19.41	179.53 ± 18.13
Carbohydrate				
kcal/day	830.00 ± 34.20	684.52 ± 25.04*[†]	764.20 ± 27.72	804.92 ± 34.12*
g/day	207.50 ± 8.55	171.13 ± 6.26*[†]	191.05 ± 6.93	201.23 ± 8.53*
% of total energy	59.33 ± 2.20	51.02 ± 1.35*[†]	52.60 ± 2.54	57.81 ± 1.62*
Sugar (g/day)	21.66 ± 2.93	21.51 ± 2.52	20.29 ± 2.59	28.27 ± 5.04
Dietary fiber (g/day)	9.00 ± 0.86	7.56 ± 0.66	8.35 ± 0.83	8.68 ± 0.70
Water (g/day)	1730.10 ± 39.85	1672.74 ± 39.11	1743.68 ± 50.45	1802.83 ± 59.74
Protein : Fat : CHO	18 : 23 : 59	24 : 25 : 51	18 : 29 : 53	17 : 25 : 58

[1] mean ± SEM
[2] Energy distribution and nutrient intakes in the WPI group included 30 g/day of WPI
* Significant difference from baseline within group ($p < 0.05$)
†Siginificant difference between groups ($p < 0.05$) ††Siginificant difference between groups ($p < 0.001$)
WPI = whey protein isolate; kcal = kilocalorie; g = gram; mg = milligram; kg = kilogram; CHO = carbohydrate

Table 11 Anthropometry of the subjects at baseline and week 6 of the experimental period [1]

Characteristics	WPI group		Control group	
	Baseline	Week 6	Baseline	Week 6
Body weight (kg)	63.24 ± 1.92	62.73 ± 1.89*	63.11 ± 2.25	62.77 ± 2.31
Height (m)	1.56 ± 0.02	1.56 ± 0.02	1.58 ± 0.02	1.58 ± 0.02
BMI (kg/m^2)	25.92 ± 0.60	25.71 ± 0.58*	25.18 ± 0.59	25.03 ± 0.60
Waist circumference (cm)	85.44 ± 1.00	84.94 ± 0.93	83.64 ± 1.96	83.62 ± 2.03
Hip circumference (cm)	96.79 ± 1.08	96.70 ± 1.13	95.16 ± 1.28	94.90 ± 1.26
WHR	0.89 ± 0.01	0.88 ± 0.00	0.88 ± 0.02	0.88 ± 0.02
TSF (mm)	16.97 ± 1.47	16.66 ± 1.43	16.27 ± 1.54	15.17 ± 1.18
MAC (cm)	30.01 ± 0.62	29.78 ± 0.57	30.24 ± 0.74	29.97 ± 0.60
MAMC (cm)	24.68 ± 0.39	24.55 ± 0.36	25.14 ± 0.66	25.21 ± 0.60

[1] mean ± SEM   * Significant difference from baseline within group ($p < 0.05$)
WPI = whey protein isolate; BMI = body mass index; WHR = waist to hip ratio; TSF = triceps skinfold;
MAC = mid-arm circumference; MAMC = mid-arm muscle circumference; kg = kilogram; m^2 = square meter;
M = meter; cm = centimeter; mm = millimeter

4.4 Anthropometric Measurement of the Subjects

The anthropometric parameters included body weight, height, BMI, waist circumference, hip circumference, WHR, TSF, MAC, and MAMC. The results are shown in **Table 11**. At baseline and week 6 of the experimental period, there were no significant differences between groups in any anthropometric parameters. However, after 6 weeks of of WPI supplementation, the mean body weight and BMI of the subjects in the WPI group were significantly less than those at baseline. The mean body weight of the subjects in the WPI group decreased from 63.24 ± 1.92 kg at baseline to 62.73 ± 1.89 kg at week 6 of the experimental period ($p = 0.043$), and BMI decreased from 25.92 ± 0.60 kg/m^2 at baseline to 25.71 ± 0.58 kg/m^2 at week 6 of the experimental period ($p = 0.043$).

4.5 Blood Pressure and Biochemical Parameters of the Subjects

Blood pressure of the subjects in the WPI group was not significantly different from that of the subjects in the control group both at baseline and week 6 of the experimental period. However, at week 6 of the experimental period, SBP in the WPI group was significantly decreased from 127.50 ± 3.19 mmHg at baseline to 118.22 ± 2.24 mmHg ($p = 0.002$).

Biochemical parameters measured in this study included FBS, HbA1c, TC, TG, albumin, and CBC (white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), neutrophil, lymphocyte, monocyte, basophil, and eosinophil). None of the biochemical parameters differed between groups and within group. However, at the week 6 of the experimental period, the TG level of the WPI group was significantly less than those at baseline. The TG level decreased from 141.89 ± 8.97 mg/l at baseline to 124.17 ± 7.54 mg/l at week 6 of the experimental period ($p = 0.043$). The results for BP and biochemical parameters are shown in **Table 12** and for CBC are shown in **Table 13**.

Table 12 Blood pressure and biochemical parameters of the subjects at baseline and week 6 of the experimental period [1]

Parameters	WPI group		Control group	
	Baseline	Week 6	Baseline	Week 6
Blood pressure (mmHg)				
SBP	127.50 ± 3.19	118.22 ± 2.24**	123.67 ± 3.97	122.33 ± 4.04
DBP	73.83 ± 1.84	77.11 ± 1.88	75.56 ± 2.46	74.44 ± 1.85
Biochemical parameters				
FBS				
mg/dl	123.39 ± 5.64	125.89 ± 4.41	114.39 ± 5.51	124.11 ± 4.87
mmol/l	6.86 ± 0.31	6.99 ± 0.25	6.35 ± 0.31	6.89 ± 0.27
HbA1c				
mg/dl	6.88 ± 0.21	6.83 ± 0.17	7.26 ± 0.37	6.97 ± 0.27
TC				
mg/dl	187.78 ± 6.67	183.39 ± 6.91	199.28 ± 6.05	199.61 ± 9.20
mmol/l	4.86 ± 0.17	4.74 ± 0.18	5.15 ± 0.16	5.16 ± 0.24
TG				
mg/dl	141.89 ± 8.97	124.17 ± 7.54*	140.72 ± 10.86	149.44 ± 10.84
mmol/l	1.60 ± 0.10	1.40 ± 0.09*	1.59 ± 0.12	1.69 ± 0.12
Albumin				
g/dL	4.58 ± 0.07	4.51 ± 0.06	4.56 ± 0.05	4.53 ± 0.07
g/l	45.78 ± 0.72	45.11 ± 0.61	45.56 ± 0.50	45.26 ± 0.65

[1] mean ± SEM
* Significant difference from baseline within group ($p < 0.05$) ** Significant difference from baseline within group ($p < 0.01$)
WPI = whey protein isolate; SBP = systolic blood pressure; DBP = diastolic blood pressure; FBS = fasting blood sugar;
HbA1c = hemoglobin A1c; TC = total cholesterol; TG = triglyceride; mmHg = millimeter of mercury;
mg/dl = milligram/deciliter; mmol/l = millimole/liter; g/dL = gram/deciliter

Table 13 Completed blood count of the subjects at baseline and week 6 of the experimental period [1]

Parameters	WPI group		Control group	
	Baseline	Week 6	Baseline	Week 6
Complete blood count				
WBC (10^3/ml^3)	6.61 ± 0.28	7.13 ± 0.29	7.11 ± 0.43	6.94 ± 0.33
RBC (10^6/ml^3)	4.23 ± 0.15	4.67 ± 0.25	4.54 ± 0.12	4.49 ± 0.11
Hb				
g/dL	11.91 ± 0.38	11.87 ± 0.40	12.68 ± 0.35	12.43 ± 0.33
g/l	119.06 ± 3.76	118.67 ± 3.98	126.78 ± 3.46	124.30 ± 3.25
Hct (%)	37.37 ± 1.09	37.35 ± 1.12	39.26 ± 0.95	38.82 ± 0.86
Neutrophils (%)	54.71 ± 1.81	56.01 ± 1.91	52.49 ± 2.44	53.33 ± 1.89
Lymphocytes (%)	34.53 ± 1.13	33.93 ± 1.49	36.74 ± 1.94	35.55 ± 1.41
Monocytes (%)	4.06 ± 0.26	4.24 ± 0.26	4.03 ± 0.29	4.18 ± 0.30
Eosinophils (%)	4.89 ± 0.80	7.56 ± 2.99	6.13 ± 1.50	5.44 ± 1.26
Basophils (%)	1.82 ± 1.30	0.64 ± 0.10	0.60 ± 0.10	0.58 ± 0.05

[1] mean ± SEM
WPI = whey protein isolate; WBC = white blood cell; RBC = red blood cell; Hb = hemoglobin; Hct = hematocrit;
10^3/ml^3 = 1,000 per cubic meter; 10^6/ml^3 = 1,000,000 per cubic meter; g/dL = gram/deciliter; g/l = gram/liter

4.6 Inflammatory Mediators in Plasma of the Subjects

Inflammatory mediators measured in this study included hs-CRP and IL-6. The results are shown in **Table 14**. The mean hs-CRP level of the subjects in the WPI group at baseline and week 6 of the experimental period were 2.32 ± 1.01 mg/l and 1.71 ± 0.36 mg/l respectively. The mean hs-CRP level of the subjects in the control group were 2.48 ± 0.50 mg/l and 3.36 ± 0.90 mg/l at baseline and week 6 of the experimental period respectively. The results demonstrated that the hs-CRP levels of the subjects in the WPI and control groups were not significantly different both at baseline and week 6 of the experimental period. However, at week 6 of the experimental period, the hs-CRP levels of the subjects in the WPI group tended to decrease from baseline (difference mean = 0.611 mg/l).

The mean plasma IL-6 level of the subjects in the WPI group at baseline and week 6 of the experimental period were 1.95 ± 0.25 pg/ml and 1.82 ± 0.20 pg/ml respectively. The mean plasma IL-6 levels of the subjects in the control group were 1.92 ± 0.17 pg/ml and 1.96 ± 0.16 pg/ml at baseline and week 6 of the experimental period respectively. There were no significance in plasma IL-6 levels between baseline and week 6 of the experimental period between groups; however, the IL-6 levels of the subjects in the WPI group tended to decrease from baselines (mean difference = 0.138 pg/ml).

Table 14 Inflammatory mediators of the subjects at baseline and week 6 of the experimental period [1]

Inflammatory mediators	WPI group		Control group	
	Baseline	Week 6	Baseline	Week 6
hs-CRP (mg/l)	2.32 ± 1.01	1.71 ± 0.36	2.48± 0.50	3.36 ± 0.90
IL-6 (pg/ml)	1.95 ± 0.25	1.82 ± 0.20	1.92 ± 0.17	1.96 ± 0.16

[1] mean ± SEM
WPI = whey protein isolate; hs-CRP = high sensitivity C-reactive protein; IL-6 = interleukin-6; mg/l = milligram/liter; pg/ml = picogram/milliliter

4.7 Compliance and Adverse Effects of Whey Protein Supplementation

Compliance of the treatment was evaluated by calculation the sachets of WPI supplement throughout the study. The average percentage of compliance was 97.04 ± 0.98.

All subjects completed the study with no serious adverse effects. The only mild adverse effect was flatulence, which was found in 3 subjects (16.67%). However, splitting dose of WPI supplementation tended to decrease the adverse effect, and it disappeared completely after the WPI supplement was stopped.