CHAPTER V

DISCUSSION

This Quasi-experimental study was conducted to investigate the effects of a whey protein supplementation on insulin resistance, clinical outcomes and adverse effects in type 2 diabetic patients.

5.1 Characteristics of the Subjects

In the present study, approximately 50% of the subjects were older than 60 years old. Fifty percent of the subjects in both groups had diabetes for less than 5 years. More than 80% of the subjects had hypertension, dyslipidemia or both. Most of them were also obese (55.6%) and had abdominal obesity (78.8%). The results were consistent with previous studies in Thai population (Aekplakorn et al., 2003; Aekplakorn et al., 2007; Rawdaree et al., 2006). Aekplakorn et al. (2003) reported that the proportion of diabetes was substantially greater for older people than younger people (63% for $\geq$ 55 years vs. 37% for $\leq$ 54 years, p = 0.01). According to the report of Thailand Diabetes Registry (TDR) Project, 58.8% of Thai diabetic population aged between 50 to 70 years. Moreover, the prevalence of dyslipidemia (73.3%), hypertension (63.3%) and obese (52.6%) were high in Thai diabetic patients, which may be associated with the high prevalence of diabetic complications (Rawdaree et al., 2006). Consistently, the data from the Third National Health Examination Survey 2004 showed that Thai diabetic patients had high levels of concomitant cardiovascular risk factors. Of those with diabetes, 72.7% had high blood pressure,

33.0% had high total cholesterol, 48.8% were overweight, and 53.5% had abdominal obesity (Aekplakorn et al., 2007).

According to the results of present study, almost all of subjects had metabolic syndrome. This finding agreed with the report that 90-95% of type 2 diabetic patients with diabetic duration less than 5 years had metabolic syndrome (Song and Hardisty, 2008). Metabolic syndrome refers to clustering of risk factors that promote the development of atherosclerotic cardiovascular disease (CVD) and its clinical role is to identify individuals at risk of cardiovascular complication. The presence of the metabolic syndrome is associated with an increased risk for cardiovascular morbidity and mortality. The presence of the metabolic syndrome in type 2 diabetic patients is associated with a higher prevalence of CVD than found in those without the syndrome (Isomaa et al., 2001; LeRoith, 2008; Song and Hardisty, 2008). Therefore, this may suggest that the subjects in the current study were at high risk of developing CVD.

5.2 Dietary and Energy Intake of the Subjects

In this study, dietary and energy intake was assessed by 3-day food records. Total energy intake in both groups were about 1,400 kcal/day without significant difference between groups at baseline and week 6. The percentage energy from carbohydrate, protein and fat consumed by the subjects in both groups was within or close to the dietary reference intake (DRI). According to the American Diabetes Association (ADA), the acceptable macronutrient distribution ranges for carbohydrate, protein and fat are 45-65%, 10-20% and 20-35% of total energy intake per day respectively. The daily intake of cholesterol should be less than

200 mg/day and 300 mg/day for diabetic patients with and without cardiovascular disease or dyslipidemia respectively. In addition, sodium intake should not exceed 2,400 mg/day. In the present study, the amounts of daily cholesterol and sodium intakes during the study met the recommendation of the ADA in both groups, which was beneficial for cardiovascular health (ADA, 2004a; Franz, 2007).

Focusing on protein intake, protein consumption at baseline was approximately 1.0 g/kg/day in both groups, which met the DRI for Thais 2003 (Nutrition division, 2003). During WPI supplementation, a significant increase in total and animal protein intake and decrease in carbohydrate intake were observed in the treatment group. In this regard, total protein intake increased to 1.3 g/kg/day, which was higher than the Thai DRI. Total protein intake of the treatment group was higher from 17.93% of total energy intake at baseline to 24.27% of total energy at week 6 of the supplementation period. These indicated that the treatment group consumed protein higher than the recommendation of the ADA during WPI supplementation period (ADA, 2004a). Animal protein intake of the treatment group was higher than those at baseline and those of the control group. These results was due to the addition of daily WPI intake (30 g/day).

5.3 Effects of WPI Supplementation on Glycemic Control and Insulin Resistance

Currently, there is not enough information about effects of whey protein on blood glucose control, insulin resistance and β-cell function in type 2 diabetic patients. After WPI supplementation for 6 weeks, there were no significant changes in values of FPG, HbA1c, serum insulin, HOMA-IR, and HOMA-B% in the treatment

and the control groups. The results indicated that 30 g daily of WPI supplementation for 6 weeks did not improve glycemic control, insulin resistance and pancreatic β-cell function in subjects with type 2 DM. The findings were similar to an open-labeled clinical study of Chitapanarux et al. (2009) that indicated that supplementation with undenatured cysteine-rich WPI (20 g/day) for 12 weeks in Thai subjects with nonalcoholic steatohepatitis (n = 38) showed no difference in fasting blood insulin levels, compared with baseline. Similarly, a randomized, double-blind, multicenter trial of Sattler et al. (2008) reported that supplementation with 20 g of whey protein twice daily for 12 weeks in HIV subjects (n = 29) had no effect on FPG, fasting blood insulin, and HOMA-IR.

In contrast, the results of the present study disagreed with some previous studies. In animal study, Belobrajdic et al. (2004) reported that a high-protein diet (32%) containing whey protein concentrate (WPC) reduced fasting blood insulin and increased insulin sensitivity in insulin-resistant Wistar rats. Royle et al. (2008) also found that feeding Wistar rats ad libitum for 7 weeks with WPI-containing diet (100 g of WPI/kg diet) produced a reduction in fasting blood insulin. Several studies in humans indicated that whey protein stimulated insulin release and reduced postprandial blood glucose excursion after meal (Calbet and MacLean, 2002; Nilsson et al., 2004; Frid et al., 2005; Kasim-Karakas et al., 2007; Petersen et al., 2009). Clinically, the postprandial blood glucose has increasingly been recognized as a highly relevant determinant of HbA1c (Fonseca, 2003). Thus, there was a hypothesis that the insulinotropic effect of whey protein might be used similarly to those pharmaceutical insulin secretagogues (such as sulfonylureas and glinides) for the purpose of facilitating normoglycemia in diabetic subjects (Frid et al., 2005).

In a long-term clinical study, meal replacements with glycomacropeptide (GMP)-enriched WPI (30 g/day for 6 months and 15 g/d for further 6 months) in overweight and obese subjects significantly reduced FPG and HbA1c levels compared with baselines (Keogh and Clifton, 2008). However, the present study demonstrated that consuming 30 g/day of WPI for 6 weeks did not reduce FPG and HbA1c levels. It was possible that the 6-week supplementation period of the present study was insufficient to reveal an effect.

In addition to the duration of WPI supplementation, there are many factors contributing to the lack of significant change in glycemic control and insulin resistance in this study. When compared the present study with the study of Keogh and Clifton (2008), there were differences in types of whey protein, dose and dosing frequency, and clinical characteristics of the subjects. In the present study, the subjects with type 2 DM were supplemented with 30 g/day of intact WPI for 6 weeks. In the study of Keogh and Clifton (2008), the healthy subjects with overweight consumed GMP-enriched WPI containing 90% GMP for 12 months. It was possible that meal replacement with GMP-enriched WPI may be more effective in improving glycemic control than meal supplementation with intact WPI. In the present study the subjects were advised to consume 30 g of WPI once daily after breakfast. However, there is limited literature in the area of whey protein and diabetes, and it was not possible at this time to estimate the effective dose and dosing frequency of whey protein. In the study of Keogh and Clifton (2008), the subjects were advised to consume the whey protein 15 g twice daily instead of two meals for 6 months and 15 g once daily instead of one meal for 6 months later. Thus, it is possible that insufficient dosing frequency was provided in this study.

Most subjects in the current study were in mild stage of DM because most of them had diabetic duration less than 5 years and none of them had chronic complication. About 56% of the subjects in the treatment group had FPG levels (less than 130 mg/dl and HbA1c less than 7 mg%) that achieved the therapeutic goals recommended by the ADA (2008). In addition, baseline serum insulin levels of all subjects in the treatment group were within the normal range (6.0-27.0 μIU/ml). The baseline HOMA-IR score in the treatment group was low. Theoretically, the higher level of fasting blood insulin and HOMA-IR means the higher degree of insulin resistance (Chanchay, 2007). The low fasting blood insulin level with normal FPG and low HOMA-IR that could be observed in the treatment group indicated that most individuals in this group were in mild stage of type 2 DM. This may suggest that short-term WPI supplementation had no beneficial effects on FPG and HbA1c levels in mild stage of type 2 diabetic patients who achieve the recommended FPG and HbA1c goals.

5.4 Effects of WPI Supplementation on Serum Lipid Profile

According to the Centers for Disease Control and Prevention (CDC), 97% of adults with diabetes have one or more lipid abnormalities (Fagot-Campagna et al., 2000). The central characteristics of dyslipidemia in type 2 diabetic patients are moderate elevation of TG levels, low HDL-C levels, and relatively normal levels of LDL-C carried in highly-atherogenic small, dense LDL particles. The presence of elevated TG and decreased HDL levels is the best predictor of CVD in patients with type 2 DM. Furthermore, diabetes seriously increases the risk of developing CVD.

Therefore, current American Diabetes Association (ADA) and National Cholesterol-Education Program (NCEP) guidelines recommend aggressive treatment of dyslipidemia in people with diabetes, particularly with elevated LDL cholesterol level (ADA, 2004b; Henry, 2001).

In the present study, total-C, HDL-C, LDL-C, and TG levels were examined in each subject at baseline and the end of the study. The results showed that there were no significant differences in total-C, HDL-C, and LDL-C levels within group and between groups. Focusing on blood cholesterol, supplementation of 30-g WPI in type 2 diabetic patients for 6 weeks did not produce the beneficial effects on total-C, HDL-C or LDL-C. The findings were consistent with a single blind, parallel study of Kawase et al. (2000) in which healthy adult men with total-C higher than 200 mg/dl consumed 200 ml of fermented milk containing WPC or placebo (casein) in the morning and evening. After the 8-week study, the results showed no change in total-C level in both groups, but the TG level was lowered, and HDL-C level was increased significantly in the WPC group. Similarly, Sattler et al. (2008) reported that supplementation of 20-g whey protein twice daily for 12 weeks in patients with HIV (n = 29) did not affect total-C, HDL-C and LDL-C levels. Tienboon et al. (2009) also reported that supplementation with undenatured cysteine-rich WPI (20 g/day) for 12 weeks had no significant effects on total-C, HDL-C and LDL-C in nonalcoholic steatohepatitic subjects with borderline-high cholesterol concentration (n = 38).

Inconsistently, several animal studies provided evidence on the cholesterol-lowering effect of whey protein. Nagaoka et al. (1991 and 1992) reported that the cholesterol lowering action of WPC was more powerful than that of casein and soy protein in animal studies. Zhang and Beynen (1993) also reported that blood and

liver cholesterol and TG levels were lower in female weaning rats fed a cholesterol-containing diet for 3 weeks after they were fed with whey protein at the amounts of 150 g/kg feed. Beena and Prasad (1997) found that standard yogurt containing condensed whey or lactose-hydrolyzed condensed whey and bifidus yogurts lowered serum total-C and LDL-C levels in albino rats, whereas whole milk and standard yogurt had no cholesterol-lowering effect. Additionally, Kawase et al. (2000) reported that WPC reduced serum total-C level in rat with the dose-dependent manner. Few human studies reported a beneficial effect of whey protein on cholesterol levels. In a randomized-controlled study of Pins and Keenan (2002), 30 pre-hypertensive or stage-1 hypertensive subjects were randomized to received 20 g/day of either hydrolyzed WPI (active treatment) or unmodified WPI (control treatment) for 6 weeks. The results indicated that LDL-C levels decreased significantly by both treatments. Keogh and Clifton (2008) reported significant reductions in total-C, LDL-C and TG in obese subjects after meal replacement with 15-30 g/day of GMP-enriched WPI for 12 months.

According to the results of the current study, TG level decreased significantly by 9.6% in the group supplemented with WPI while that did not change significantly in the control group. Moreover, the TG level showed a high tendency to be lower in the treatment group compared to the control group. These results agreed with previous studies, which demonstrated that intake of whey protein produced a significant TG-lowering effects (Sattler et al., 2008; Keogh and Clifton, 2008). Sattler et al. (2008) found that fasting TG level decreased by 16 ± 62 mg/dl in the patients with HIV receiving a 280-kcal supplement containing 20 g of whey protein twice daily for 12 weeks but increased by 39 ± 98 mg/dl in the control group receiving an isocaloric

control supplement containing casein. Other lipids did not change significantly. Consistently, Keogh and Clifton (2008) found that meal replacements with 15 g of GMP-enriched WPI once to twice daily for 12 months produced a significant decrease in TG level (26.6 ± 4.3 mg/dl, $p < 0.001$) in overweight or obese subjects. However, the reduction in TG level for the WPI group did not differ from that of the control group receiving an isocaloric control supplement containing skim milk powder.

Interestingly, the results of the present study indicated that the subjects with baseline TG higher than 150 mg/dl in the treatment group had significant greater reduction of TG than those with TG lower than 150 mg/dl. These results provided initial evidence suggesting that WPI consumption may help to prevent the development of CVD by controlling TG level in type 2 diabetic patients, especially in individuals who fail to achieve the treatment goals for TG.

The mechanisms of lipid improvement were not revealed in the present study. To date, little research has been done on whey protein's effect on lipid profile. Thus, the mechanism relating TG-lowering effect of whey protein is still not well understood. According to a crossover study in 12 patients with type 2 DM (Mortensen et al., 2009), the results suggested that whey protein seemed to outperform other proteins in terms of postprandial lipidemia improvement, possibly because of the formation of fewer chylomicrons or increased clearance of chylomicrons. In the study of Mortensen et al. (2009), blood samples were collected over 8 hours after ingestion of a test meal containing 100 g butter and 45 g carbohydrate in combination with 45 g casein (casein-meal), whey (whey-meal), cod (cod-meal), or gluten (gluten-meal). The incremental area under the curve for TG was significantly lower after ingestion of

the whey-meal than the other meals. The levels of free fatty acids were most pronouncedly suppressed after ingestion of the whey-meal.

5.5 Effects of WPI Supplementation on Other Biochemical Parameters

Blood biochemical parameters including albumin, uric acid, SCr, AST, ALT, ALP, BUN, and electrolytes of the subjects were measured at the beginning and the end of the study to investigate the effect of WPI supplementation. The results indicated that these parameters did not change significantly from baseline, and no relevant difference was found between groups, except for ALP and BUN. In the treatment group, mild elevated ALP level was found, but all values were within normal range (39-117 U/L). Clinically, mild elevation of ALP (up to 1.5 times normal) can be found in elderly patients without an underlying pathologic condition (Flora and Keeffe, 1990; Trombetta and Foote, 2009). Focusing on renal function test, the BUN and SCr levels are useful parameters in assessing renal function. At week 6 of the WPI supplementation period, mean BUN level in the treatment group was higher than those at baseline and those of the control group. However, an increase in BUN above the normal range was found only in three subjects. SCr level which is a more specific and sensitive indicator of renal function than BUN was within normal range (0.5-1.8 mg/dl) in all subjects receiving WPI. If BUN raises and SCr level remains stable, this is likely due to protein loading rather than reduced renal function (Bellack and Edlund, 1992; Trombetta and Foote, 2009). The results of this study indicated that the treatment group consumed high amount of protein (1.32 g/kg/day or 24.27% of total daily energy) during the WPI supplementation. Therefore, elevated BUN in the treatment group can be attributed to protein loading.

There are some concerns about kidney and liver function that the decline in these organ functions may occur when large amount protein are consumed. However, there is no scientific evidence showing that high-protein intake has adverse effects on liver and renal function (Manninen, 2004). According to previous clinical studies, abnormalities in liver and renal function were not found after daily whey protein supplementation in the amount of 20-60 g for 1-12 months (Frestedt et al., 2008; Grey et al., 2003; Kasim-Karakas et al., 2009; Keogh and Clifton, 2008; Marshall, 2004; Micke et al., 2001 and 2002; Pins and Keenan, 2002; Sattler et al., 2008; Sinnott et al., 2009; Tienboon et al., 2009).

The present study provided initial evidence suggesting that whey protein consumption or high-protein intake may be associated with elevated BUN level in type 2 diabetic patients. According to the recommendations of the ADA, type 2 diabetic patients with normal renal function should consume protein 10-20% of total daily energy to avoid renal hyperfiltration that may lead to renal impairment. Intake of protein in the usual range does not appear to be associated with the development of diabetic nephropathy (ADA, 2004a). Further studies are necessary to determine the total magnitude of response and adverse effects of long-term supplementation of whey protein.

5.6 Effect of WPI Supplementation on Blood Pressure

Although the results of this study indicated that there were no differences in SBP and DBP between the treatment and the control groups, a significant reduction in SBP was observed in the treatment group. These results confirmed several previous human studies that demonstrated a hypotensive effect of whey protein and fermented

dairy products containing whey protein in both pre-hypertensive and hypertensive subjects. A clinical study investigated the effect of fermented milk supplemented with WPC on blood pressure in healthy men (Kawase et at., 2000). SBP was reported to be slightly lowered in the group receiving the fermented milk for 8 weeks (400 ml/day) as compared with that group receiving a control fluid containing casein. Pins and Keenan (2002) found that daily supplementation of 20-g hydrolyzed WPI for 6 weeks in untreated borderline to mild hypertensive adults (BP>120/80 mmHg and <155/95 mmHg) was associated with an average SBP decrease of 8.0 ± 3.2 mmHg and an average drop in DBP of 5.5 ± 2.1 mmHg, compared with WPI.

Consistently, Keogh and Clifton (2008) reported that meal replacements with 15 g of GMP-enriched WPI once to twice daily for 12 months produced significant decrease in SBP and DBP by 8 ± 17 and 5 ± 12 mmHg respectively. Pal and Ellis (2009) evaluated the effects of whey protein supplementation on blood pressure, vascular function and inflammatory markers compared to casein and glucose (control) supplementation in overweight or obese individuals. They found that SBP decreased significantly at weeks 6 and 12 compared to baseline in the whey and casein groups. DBP decreased significantly in the whey and casein groups compared to baseline. In addition, a meta-analysis of 15 placebo-controlled clinical trials showed that peptides derived from food proteins (fermented milk, whey protein, casein, fish) could significantly reduce SBP and DBP with pooled mean effects of -5.13 mmHg (95% CI: -7.12, -3.14) and -2.42 mmHg (95% CI: -3.82, -1.03) respectively (Pripp, 2008).

Currently, the mechanism underlying the hypotensive effects of whey protein are not fully understood. Several studies suggested that whey protein contains a number of bioactive peptides such as angiotensin I converting enzyme (ACE)-

inhibitory peptides and opiod-like peptides. The bioactive peptides may act either indepentdently or synergistically to reduce blood pressure (FitzGerald et al., 2004, Jauhiainen and Korpela, 2007, Mullally et al., 1997; Pihlanto-Leppala et al., 2000). These peptides are inactive within the sequence of the precursor protein, but they can be released by enzymatic proteolysis either during gastrointestinal digestion or during food processing. Once the peptides are liberated from proteins, they may exhibit the ACE-inhibitory activity. To exert a hypotensive effect after oral ingestion, these peptides have to reach the cardiovascular system in an active form. Therefore, they need to remain active during digestion by gastrointestinal proteases, pass from intestinal wall into the blood, be resistant to degradation by serum peptidases, and reach target organ (FitzGerald et al., 2004; Vermeirssen et al., 2004).

In vitro, incubation of milk proteins with gastrointestinal proteinases such as trypsin, pepsin and chymotrypsin resulted in the release of ACE inhibitory peptides (FitzGerald et al., 2004; Meisel and Bockelmann, 1999). During in vitro studies with the whey protein-derived peptide (β-lactoglobulin f(142-148)), Vermeirssen et al. (2002) demonstrated that this peptide could be transported intact through a human intestinal Caco-2 cell monolayer. Short peptides appeared in the blood as a result of paracellular transport of short peptides though the intercellular junction, but larger peptides (more than 4 residues) could be transported via carriers (Satake et al., 2002; Shimizu et al., 1997). Thus, different results among clinical studies that investigated hypotensive effect of whey protein may be due to variability in digestion and absorption of whey protein and its bioactive peptides.

It is well established that elevated blood pressure is an independent risk factor for both microvascular and macrovascular complications of diabetes, particularly

CVD. Blood pressure control has been shown to reduce the risk for CVD. In the UK Prospective Diabetes Study, each 10-mmHg reduction in SBP was associated with a reduction of 12% in any diabetes-related complication, 15% in diabetes-related dealth, 11% in myocardial infarctions, and 13% in microvascular complications (Leroith, 2008). In the present study, there was a mean reduction of 9 mmHg in SBP in the treatment group. Therefore, supplementation of whey protein in type 2 diabetic subjects, may be helpful in achieving the treatment goals for blood pressure, resulting in a reduction of the risks for CVD and other diabetes-related complications. However, further research are necessary to confirm these findings.

5.7 Effect of WPI Supplementation on Anthropometric Parameters

In the present study, daily supplementation of 30-g WPI for 6 weeks resulted in a significant decrease in body weight and BMI in the treatment group, whereas no significant change in any anthropometric parameters was observed in the control group. The mean weight loss were 0.52 kg, which represented a 0.80% decrease from baseline, and the mean reduction of the BMI was 0.21 kg/m^2. Moreover, WC which is a simple anthropometric index of abdominal visceral adipose tissue accumulation and related cardiovascular risk, tended to decrease from baseline in the treatment group. These findings were consistent with previous clinical trials in overweight or obese subjects that showed decreased body weight and improved body composition after daily supplementation of 20-60 g of whey protein for 4 weeks to 12 months (Baer et al., 2006; Frestedt et al., 2008; Keogh and Clifton, 2008; Sinnott et al., 2009; Tienboon et al., 2009).

Baer et al. (2006) studied the effects of daily supplementation with 60-g whey protein for 6 months, compared to soy protein and carbohydrate, on body weight and composition in free-living, overweight or obese healthy individuals. They found that body weight and body fat (measured by bioelectrical impedance) of the group consuming whey protein was lower than those of the carbohydrate treatment group ($p < 0.006$). Moreover, WC was lower ($p < 0.0001$) in the whey protein group than the two other groups. Frestedt et al. (2008) studied in obese subjects adhering to a 500 calorie-reduced diet and consuming 20 g daily of WPI or an isocaloric beverage (control) for 12 weeks. The results demonstrated that whey protein significantly decreased body weight and body fat from baselines. More recently, supplementation with 24.4 g/day of specialized whey protein (contain 20 g of protein and 488 mg of calcium) in overweight individuals resulted in a steady loss of body weight, decrease in BMI and WC at weeks 4 and 8 after initiating the supplement (Sinnott et al., 2009). A pilot study in Thai patients with nonalcoholic steatohepatitis reported that daily supplementation of 20 g undenatured, cysteine rich WPI (HMS 90) for 12 weeks leaded to a significant reduction of body weight, BMI and WC (Tienboon et al., 2009). Keogh and Clifton (2008) studied in obese subjects consuming GMP-enriched WPI or skim milk powder (30 g/day instead of 2 meals for 6 months and 15 g/day for a further 6 months) and found that weight reduction was about 10-11 kg in both groups at months 6 and 12 compared to baseline.

To date, the exact mechanisms by which whey protein intake impacts on weight loss is not known. There is an evidence suggesting that whey protein contains a high proportion of bioactive compounds such as ACE-inhibitory peptides, branched chain amino acids, and calcium, which may act either independently or synergistically

to enhance body weight loss, body fat loss, and maintain lean muscle mass (Zemel, 2005).

In addition to bioactive compounds in whey protein, it is possible that an increase in protein intake and/or decrease in carbohydrate intake may be responsible for the decreases in body weight and TG observed in this study. Some studies have suggested that higher protein diets may increase total weight loss and the percentage of fat loss (Halton and Hu, 2004; Layman et al., 2003). In the study of Layman et al. (2003), obese females were assigned to either a carbohydrate group consuming a diet with a carbohydrate/protein ratio of 3.5 (68 g protein/day) or a protein group with a ratio of 1.4 (125 g protein/day). After consuming the diets for 10 weeks, the two groups lost 7.0-7.5 kg body weight. Weight loss in the protein group was partitioned to a significantly higher loss of fat, compared with the carbohydrate group. The protein group also had significant reduction in TG level (21%). In a 6-month randomized trial of overweight and obese subjects, fat loss was almost twice as great in the subjects receiving a high-protein diet (25% of total energy; 128-139 g/day), compared with a moderate-protein diet (12% of total energy; 76-80 g/day) (Due et al., 2004). The mechanisms by which increased protein intake impacts on weight and fat loss are not clear. There is an evidence suggesting that higher protein intake can enhance thermogenesis compared to diets lower in protein content, resulting in increased energy expenditure (Halton and Hu, 2004; Paddon-Jones et al., 2008).

It is generally accepted that both obesity and DM increase the risk of CVD. Therefore, weight loss should be a primary recommendation for overweight type 2 diabetic patients. Clinically important weight reduction is defined as reduction of 5–10% of initial body weight. Long-term studies have shown that a sustained

moderate weight loss of 5-10% improves insulin sensitivity, blood glucose, lipid, and blood pressure levels, with potential reductions in cardiovascular risk. Weight reduction also appears to prolong survival in obese individuals with type 2 DM (Goldstein, 1992; Goodpaster et al., 1999: Lean et al., 1990; McLaughlin et al., 2001; Pasanisi et al., 2001). In the present study, weight reduction in the treatment group did not achieve the weight reduction goal (0.5-1 kg/week or a reduction of 5-10% of initial body weight) after 6 weeks of the WPI supplementation. Further investigations should find whether long-term supplementation of whey protein could achieve the goal.

5.8 Adverse Effects and Tolerance of Whey Protein

In the present study, no serious adverse event was observed during the study. WPI was well tolerated and no subject withdrew from the study due to adverse events. The study results were consistent with several clinical studies showing that whey protein was generally well tolerated and severe adverse events did not occur (Frestedt et al., 2008. Grey et al., 2003; Kasim-Karakas et al., 2009; Keogh and Clifton, 2008; Micke et al., 2001 and 2002; Pins and Keenan, 2002; Sattler et al., 2008; Sinnott et al., 2009; Tienboon et al., 2009). However, 3 of 18 subjects (16.67%) in the treatment group reported mild flatulence. It was consistent with other clinical trials in healthy subjects and patients with disease states that reported the most common mild side effect of whey protein was gastrointestinal disturbance such as bloating and flatulence (Micke et al., 2001; Micke et al., 2002; Sattler et al., 2008; Sinnott et al., 2009; Tienboon et al., 2009).