CHAPTER V

DISCUSSION

The present study was conducted to study the effect of whey protein on inflammatory mediators in type 2 DM at Public Health Center 66, Bangkok Metropolitan Administration.

5.1 Baseline Inflammatory Mediators of the Subjects

The inflammatory mediators in the present study included hs-CRP and IL-6. The mean hs-CRP levels at baseline in the WPI (2.32 ± 1.01 mg/l) and control (2.48 ± 0.50 mg/l) groups appeared to be similar to the results by Fröhlich (2000) who found that the mean hs-CRP concentrations in diabetic patients were 2.03 mg/l (1.34 mg/l in healthy subjects). The hs-CRP is a protein that travels through the blood from sites of injury or infection during the acute phase response of inflammation (Kindt, 2007), and median normal concentration of hs-CRP is 0.8 mg/l (Reeves, 2007). Several studies showed that plasma concentration of inflammatory mediators increased during insulin resistance state in obese and type 2 DM patients (Pickup et al., 1997; Ford, 1999; Pickup et al., 2000; King et al., 2003). The epidemiologic studies in primary prevention have demonstrated that a single measurement of hs-CRP is a strong predictor of future vascular events and is independent of the traditional cardiac risk factors (Heber, 2008). The mean hs-CRP level at baseline of diabetic patients in the present study was in the range of 1 to > 3 mg/l, determining intermediate to high cardiovascular risk according to the American Heart Association recommendation (Heber, 2008).

In this study the plasma IL-6 levels in the WPI and control groups at baseline were 1.95 ± 0.25 pg/ml and 1.92 ± 0.17 pg/ml respectively. The IL-6 is a multi-functional cytokine acting on many cells and tissue, and circulating IL-6 level is generally ≤ 4 pg/ml (Carey and Febbraio, 2004; Kim et al., 2009). The levels of IL-6 in the present study were similar to the results from the previous studies. Pickup et al. (2000) showed that IL-6 in plasma from diabetic patients were 1.8 pg/ml. Kado et al. (1999) also reported that the serum IL-6 level in diabetic subjects was significantly higher than those in normal healthy controls (3.48 ± 3.29 pg/ml vs. 0.784 ± 0.90 pg/ml). Tan et al. (2004) determined that the hs-CRP and IL-6 levels were significantly increased in diabetic patients compared with the healthy subjects.

This study found that the hs-CRP levels significantly correlated with IL-6 in the subjects at baseline. The results were consistent with the study of Tan et al. (2004) determining that the log(hs-CRP) in diabetic patients were significantly correlated with log(IL-6). Pickup et al. (2000) also indicated significant correlation between hs-CRP and IL-6, hs-CRP and body weight, and hs-CRP and BMI. Yanagawa et al. (2007) investigated serum hs-CRP and IL-6 levels in Japanese type 2 DM, and they found that hs-CRP levels were significantly correlated with IL-6, BMI, DBP, and TG. However, the present study did not found the correlations between hs-CRP and body weight, BMI, DBP, and TG.

5.2 Dietary Intake of the Subjects

After whey protein supplementation, the amount of total protein and protein from animal consumption were significantly increased. The results from this study agreed with the previous studies, which found that the protein intake and percentage of calories from protein intake were significantly increased during whey protein supplementation (Frestedt et al., 2008; DeNysschen, 2009). The percentage of calories from total protein intake after whey protein supplementation in the present study (24% of total energy) was slightly higher than that recommended by the American Diabetes Association (ADA, 2008) for diabetic patients with normal renal function (15-20% of total energy).

This study found significant change in carbohydrate intake in both groups after the experimental period, but it was still within the range recommended by ADA (45-65% of total energy). The intakes of fat ($\leq$ 30 % of total energy) and cholesterol (< 200 mg/day) also achieved ADA recommendation even though cholesterol intake in the control group at baseline was slightly higher than ADA recommendation (ADA, 2008).

5.3 Effect of Whey Protein Supplementation on Inflammatory Mediators

After whey protein supplementation for 6 weeks, the hs-CRP levels tended to decrease, and the IL-6 also tended to decrease even though significant difference within group and between groups were not shown. The results were similar to the previous study of Lee et al. (2007) that reported no significant changes in circulating levels of hs-CRP and IL-6 after consuming 125 ml of milk drink supplemented with whey peptides for 12 weeks in hypertensive patients. Similarly, Ballard et al. (2009) found that healthy men

and women supplemented with 5 g novel whey-derived peptide for 2 weeks had significantly improved flow mediated dilation responses and increased hyperemia, compared to the baselines. There were no changes in BP, FBS, and hs-CRP in these subjects when compared with placebo. In contrast, in vitro study presented that Enprocal™ (composition: WPC 41.4%, inulin, minerals, and vitamins), digested Enprocal™, and WPC 80 down-regulated the pro-inflammatory cytokines secretion and reduced the level of LPS stimulated IL-6 (Kanwar et al., 2009). Oben et al. (2008) found that healthy males who consumed 50 g of WPC containing either 2.5 g or 5 g of a proteolytic enzyme for 9 days had significant increase in amino acid levels and nitrogen excretion, and decrease in hs-CRP levels compared to the control group (50 g of WPC).

The hs-CRP and IL-6 levels appeared to be dependent on the characteristics and conditions of individuals (Ford, 1999; Heilbronn et al., 2002; Pearson et al., 2003; Fredrikson et al., 2004; Kimberly et al., 2006; Libra et al., 2006; Natali et al., 2006; Rekeneire et al., 2006; Fischer et al., 2007). The levels of these parameters were elevated in the individuals who had high levels of FBS, insulin resistance, BP, TC, TG, and BMI (Ford, 1999; Heilbronn et al., 2002; Pearson et al., 2003; Fredrikson et al., 2004; Kimberly et al., 2006; Libra et al., 2006; Natali et al., 2006; Rekeneire et al., 2006; Fischer et al., 2007). Moreover, many presented features of metabolic syndrome, cigarette smoking, estrogen/progestogen hormone use, physical inactivity, complications of diabetes mellitus, duration of diabetes mellitus, chronic infection, and chronic inflammation were also associated with elevated hs-CRP and IL-6 levels (Ford, 1999; Heilbronn et al., 2002; Pearson et al., 2003; Fredrikson et al., 2004; Kimberly et al.,

2006; Libra et al., 2006; Natali et al., 2006; Rekeneire et al., 2006; Fischer et al., 2007). In contrast, the hs-CRP and IL-6 levels were low in the individuals with weight loss and endurance exercise (Orchard et al., 2005). The anti-inflammatory agents and statins use were associated with low hs-CRP and IL-6 levels as well (Hansson et al., 1999; Hundal et al., 2002; Haffner et al., 2002; Sjöholm and Nyström, 2006).

In the present study, the parameters measured including body weight, BMI, BP, and TG were significantly decresased after WPI supplementation, but they did not significantly differ from the control group. The changes in these parameters may be the results of WPI supplementation that in turn may affect the inflammatory mediators. The significant decrease in body weight after WPI supplementation for 6 weeks in this study agreed with the animal study of Belobrajdic et al. (2004) which indicated that obese rats fed with whey protein concentrate for 6 weeks reduced body weight gain by 4%, compared to those fed with red meat. Huang et al. (2008) demonstrated that whey protein stabilized weight gain and had the strongest satiety effect when compared with soy protein, red meat and milk protein in obese mice. Similarly, Frestedt et al. (2008) reported that the obese subjects (BMI 30-42 kg/m^2) tended to lose weight and body fat more than the control group after consuming a 20 g/day of whey fraction (high in leucine, bioactive peptides, and milk calcium) for 12 weeks.

The effect of whey protein on weight reduction may be the results of high protein consumption. There was a study revealed that overweight and obese subjects who consumed high protein diet (25% of total energy) achieved a greater weight loss than those who consumed medium protein diet (12% of total energy) (Due et al., 2004).

Clifton et al. (2008) reported that the obese subjects who consumed diet high in protein (88 g protein/day) had weight loss more than those who consumed diet low in protein, and protein intake evaluated from dietary records was directly related to weight loss. Consistently, Noakes et al. (2005) found that obese women who consumed high protein and low fat diet (31.3% protein and 22.1 % fat) for 12 weeks had weight loss by 7.3 kg. Besides the amount of whey protein consumed, bioactive components in whey protein may also play a role in weight loss. It has been shown that glycomacropeptide, the peptide found in whey protein, stimulated release of cholecystokinin which may promote satiety hormone (Degen et al., 2001; Royle et al., 2008). Therefore, weight reduction found in this study may also be the result of such peptide components in whey protein.

Hypertension is an extremely common co-morbidity of diabetes, and the prevalence of hypertension is 1.5-3.0 times greater in the diabetic patients compared with matched non-diabetic individuals (Wingard and Barrett-Connor, 1995; Simonson, 1998). In this study, the SBP was significantly decreased after 6-week whey protein supplementation. The finding was similar to the study of Pins and Keenan (2008) which indicated that the 30 prehypertensive or stage 1 hypertensive subjects had significantly decreased in SBP and DBP from baseline after supplementation with unhydrolyzed whey protein isolate in the amount of 20 g/day for 6 weeks. In contrast, Lee (2007) found that the resting SBP and DBP of hypertensive subjects who consumed whey peptides (125 ml/day of a drink containing 2.6% protein equivalent) for 12 weeks did not change. The different results found in these studies may be due to the differences in dosage and type of whey protein supplementation.

The blood pressure may be the result of the peptide components in whey protein. After proteolysis with different digestive enzymes, several peptides were released. These peptides including α-LA and β-LG performed ACE inhibitory activity (Mullally et al., 1997a; Mullally et al., 1997b; Abubakar et al., 1998; Pihlanto-Leppälä, 2001; Murakami et al., 2004; Vermeirssen et al., 2004; Costa et al., 2005). Previous study in spontaneously hypertensive rats (SHR) reported the hypotensive effect of whey-derived peptides (Abubakar et al., 1998). The depressive effect on the SBP was observed at 6 hours after gastric intubation of 8 mg/kg whey protein that was digested by proteinase K. Costa et al. (2005) evaluated the effect of the intraperitoneal (ip) administration of a whey protein hydrolysate (WPH) digested with proteinase on SBP in SHR. They also found that after 2 hours of WPH administration at the dose of 0.5 g/kg and 1 g/kg, lower SBP was observed in SHR, compared to the control group.

The present study showed that supplementation of 30 g WPI in type 2 diabetic patients for 6 weeks produced significantly decrease in serum TG. High total serum lipid levels are associated with higher risk of coronary disease (Kumar and Sivakanesan, 2009). It is widely accepted that the supplementation of a lipid-lowering agent in combination with diet and exercise would be preferential to the use of lipid-lowering medication (Morris and FitzGerald, 2008). The TG-lowering effect of whey protein found in this study was consistent with the previous studies. Zhang and Beynen (1993) found that the rats fed with whey protein diet (300 g protein /kg feed) for 3 weeks had significantly lowered plasma TC, liver cholesterol and plasma TG compared to those fed with high casein diet. Pilvi et al. (2007) also determined that obese mice fed with whey

protein isolate-based high calcium diet had significantly reduced hepatic lipid accumulation and lipid droplet size. Likewise, DeNysschen (2009) revealed that supplementation with 26.6 g of whey protein daily for 12 weeks in the overweight male subjects (BMI 25-30 kg/m^2) with TC more than 200 mg/dl tended to decrease TG levels more than soy supplementation.

There was a study reported that the LDL-C, HDL-C, and TG were decreased with weight loss. Noakes et al. (2005) found that obese women who consumed high protein, low fat diet for 12 weeks could reduce weight, LDL-C, HDL-C, and TG concentrations more than those who consumed high carbohydrate, low fat diet. In addition, Clifton et al. (2008) demonstrated that obese women received high protein diet (34% of total energy) for 12 weeks lost weight by 4.6 ± 5.5 kg, and plasma cholesterol and triglyceride improved with weight loss without significant difference from those who consumed high carbohydrate diet (64% of total energy). Thus, the decrease in TG level in the present study may be due to the significant decrease in body weight after whey protein supplementation. No change in the TC level from baseline in WPI group. In addition, the decrease in TG level in the present study may be the result of the peptide components in whey protein. Yamauchi et al. (2003) found that the β-lactotensin, the peptide component found in whey protein also exhibited hypocholesterolemic activity in mice.

In this study, after whey protein supplementation for 6 weeks both hs-CRP and IL-6 levels were not significantly changed even though decreased blood pressure was found. The result disagreed with Pins and Keenan (2008) who tested BP lowering effect

of hydrolyzed whey protein supplementation in prehypertensive or stage 1 hypertensive subjects. They found that hs-CRP level was significantly improved along with BP reduction after 20 g/d of hydrolyzed whey protein treatment for 6 weeks. One of the possible reasons that made the result different between the present and previous studies was subject characteristics. Most of the subjects in the present study were in mild stage of type 2 DM as all of them took only sulfonylurea and/or biguanide, and HbA1c reached the goal recommended by the ADA (less than 7 mg%). More than 50% of the subjects had duration of diabetes less than 5 years. All of them had baseline BP less than 160/100 mmHg without other chronic complications. The IL-6 levels of these subjects were within normal range ($\leq$ 4 pg/ml). Therefore, the proinflammatory mediator lowering effects of whey protein may not be clearly pronouced in these subject conditions. In addition, most of the studies that investigated the effect of other dietary supplements such as alpha tocopherol, flaxseed-derived lignan, and soy protein on inflammation mediators usually took about 8-12 weeks (Devaraj and Jialal, 2000; Pan et al., 2007; Hall et al., 2005). Hence, it is also possible that the 6-week WPI supplementation period in the present study may not be sufficient enough to reveal the significant effect of WPI on immune system.

Currently, human studies demonstrating the anti-inflammatory effect of whey protein are limited, and the lowering effects of whey protein on inflammatory mediators remain poorly understood. There was study reported that the IL-6 activated the hs-CRP production from the liver, and hs-CRP level was a direct indicator of IL-6 level in vivo (Bataille et al., 1992; Bastard et al., 2006). In addition, approximately 10-35% of serum

IL-6 originated from adipose tissue (Febbraio and Pedersen, 2002), and the IL-6 secretion from subcutaneous fat was in proportion to fat mass (Mohamed-Ali et al., 1997). In the present study, the mean body weight and BMI were significantly decreased, and the levels of hs-CRP and IL-6 tended to be decreased after whey protein supplementation for 6 weeks. Thus, the mechanism of whey protein supplementation in modulating inflammatory mediators may involve weight reduction. It was consistent with the study of Heibroon et al. (2001) that showed the reduced hs-CRP concentrations in healthy obese women by 26% and weight loss by 7.9 kg after 12 weeks of energy restriction. Similarly, Tchernof et al. (2002) demonstrated that hs-CRP values were decreased by 32% in obese postmenopausal women who lost 15.6% of their body weight. The hs-CRP changes were correlated with changes in body weight and fat mass. Other studies found that the hs-CRP level was reduced in obese women with weight loss after consumption of high protein diet (Noakes et al., 2005; Clifton et al., 2008).

The effect of whey protein supplementation on inflammatory mediators may also result from the influence of peptides in whey protein, particularly lactoferrin (LF). Mattsby-Baltzer et al. (1996) found that LF had an anti-inflammatory activity. They revealed that human milk LF, bovine LF, and lactoferricin B (a bactericidal pepsin-derived fragment of bovine LF) could suppress the IL-6 release from monocytic cell line (THP-1) when stimulated by lipopolysaccharide (LPS). Consistently, Håversen et al. (2002) showed that LF down regulated the LPS-induced cytokine production in human monocytic cells.