CHAPTER II

LITERATURE REVIEW

2.1 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus (type 2 DM) is a metabolic disorder characterized by hyperglycemia and accounts for 90-95% of those with diabetes. Type 2 DM is a progressive disease that, in many cases, is present long before it is diagnosed. The hyperglycemia develops gradually and patient may or may not experience the symptoms of hyperglycemia. The risk of developing type 2 DM increases with age, obesity, and lack of physical activity. It occurs more frequently in individuals with a family history of DM, individuals with hypertension or dyslipidemia, women with prior gestational diabetes mellitus, and its frequency varies in different racial/ethnic subgroups (Franz, 2007; American Diabetes Association, 2010b).

Type 2 DM has become a significant public health concern because it is a major cause of premature morbidity and mortality, particularly from CVD. In many instances, type 2 DM is considered to be one component within a group of disorders called the metabolic syndrome. Metabolic syndrome, also called insulin resistance syndrome, is a cluster of cardiovascular risk factors including abdominal obesity, dyslipidemia (elevated LDL-C, elevated TG, and low HDL-C), elevated blood pressure, insulin resistance, and prothrombotic and proinflammatory states. Each component of the syndrome is associated with an increased risk of CVD. Therefore, the management of type 2 DM must address not only the control of hyperglycemia, but also the other cardiovascular risk factors such as dyslipidemia, insulin resistance,

hypertension and obesity (Grundy et al., 2005; Reaven, 2005; Tenenbaum et al., 2003).

2.2 Pathophysiology of Type 2 Diabetes Mellitus

Type 2 DM results from a combination of insulin resistance (decreased tissue sensitivity or responsiveness to insulin) and pancreatic β-cell failure (decreased insulin secretion). However, type 2 diabetic patients usually have relative rather than absolute insulin deficiency. In the early stage of type 2 DM, the predominant abnormality is insulin resistance. Insulin resistance is first demonstrated in target tissues, mainly muscle, liver, and adipose cells. Initially there is a compensatory increase in insulin secretion, which maintains normal glucose concentrations; but, as the disease progresses, insulin production gradually decreases. Glucose abnormalities are first demonstrated by postprandial hyperglycemia, which is caused by the loss of first-phase insulin secretion and reduced suppression of hepatic glucose output after meals due to insulin deficiency and glucagon excess. As insulin secretion decreases, hepatic glucose production increase, causing the increase in fasting blood glucose levels. (LeRoith et al., 2003; Franz, 2007; American Diabetes Association, 2010b).

It is now understood that several hormones have roles in maintaining glucose homeostasis. Amylin and incretin hormones (glucagon-like peptide 1, glucose-dependant insulinotropic polypeptide) are now recognized as influential factors in maintaining glucose homeostasis. Adipose tissue also has an important role in the pathogenesis of type 2 DM. Insulin resistance at the adipocyte level leads to lipolysis and an elevation in circulating free fatty acids. Particularly, excess abdominal obesity (characterized by an excess accumulation of visceral fat around and inside abdominal organs) results in an increased flux of free fatty acids to the liver, leading to an

increase in insulin resistance. Moreover, this increase in free fatty acids also causes a further decrease in insulin sensitivity at the cellular level, diminishes the skeletal muscle insulin response, impair pancreatic insulin secretion, and augment hepatic glucose production. All these defects contribute to the development and progression of type 2 DM and are also primary targets for pharmacologic therapy (AACE Diabetes Mellitus Clinical Practice Guidelines Task Force, 2007; Franz, 2007).

2.3 Diagnosis of Diabetes Mellitus

The diagnosis of diabetes mellitus (DM) is based on blood testing, which should use venous samples, not capillary (Asian-Pacific Type 2 Diabetes Policy Group, 2005). The criteria for the diagnosis of DM described by the American Diabetes Association (ADA) are shown in **Table 1**. There are four ways to diagnose DM. Each must be confirmed on a subsequent day unless unequivocal symptoms of hyperglycemia are present. Hyperglycemia, not sufficient to meet the diagnostic criteria for DM, is categorized as either impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). IFG and IGT have been officially termed "pre-diabetes". Both categories of pre-diabetes are risk factors for DM and CVD (ADA, 2010a; Franz, 2007).

Table 1 Diagnosis of diabetes and impaired glucose homeostasis

Diagnosis	Criteria
Diabetes[a]	
1) Hemoglobin A1c (HbA1c)[b] ≥ 6.5% or	
2) Fasting plasma glucose (FPG)[c] ≥ 126 mg/dl (7.0 mmol/l) or	
3) 2-h Plasma glucose (2hPG) ≥ 200 mg/dl (11.1 mmol/l) during OGTT[d] or	
4) Random plasma glucose ≥ 200 mg/dl (11.1 mmol/l), in a patient with classic symptoms of hyperglycemia.	
Pre-diabetes	
Impaired fasting glucose	FPG 100-125 mg/day (5.6-7.0 mmol/l)
Impaired glucose tolerance	2hPG 140-199 mg/day (7.8-11.0 mmol/l)
Normal	FPG < 100 mg/day (5.6 mmol/l)
	2hPG <140 mg/day (7.8 mmol/l)

Source: Adapted from the American Diabetes Association (2010a)

[a] In the absence of unequivocal hyperglycemia, criteria 1) – 3) should be confirmed by repeat testing.

[b] The test should be performed in a laboratory using a method that is the National Glycohemoglobin Standardization Program certified and standardized to the Diabetes Control and Complications Trial reference assay.

[c] Fasting is defined as no caloric intake for at least 8 hours

[d] The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

2.4 Epidemiology of Diabetes Mellitus

DM is a large and growing global health problem. The number of patients with DM is increasing rapidly. According to the estimation of the WHO, the prevalence of DM for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of patients with DM is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild et al., 2004). The greatest increase is projected for

economically developing countries. In South-East Asia region, the number of patients with DM is projected to increase almost threefold over this time period from 46.9 to 119.5 million. Although the prevalence of both type 1 and type 2 DM is increasing worldwide, the prevalence of type 2 DM is rising much more rapidly because of increasing obesity, reduced physical activity, population growth, and aging as countries become more industrialized (O'Connell, 2005; Powers, 2008).

For Thailand, the number of patients with DM is projected to gradually increase from 1.54 in 2000 to 2.74 million in 2030 (WHO, 2008). The estimated national prevalence of DM in Thai adults aged ≥ 35 years is 9.6% (2.4 million people), which includes 4.8% previously diagnosed and 4.8% newly diagnosed (Aekplakorn et al., 2003). Besides, the 2003-2004 health examination survey on Thai people revealed that the prevalence of DM had risen from 2.3% in 1991 to 4.6% in 1996 and 6.9% or 3.2 million individuals in 2004. This is evident that the prevalence of DM in Thailand has a rising trend; and more importantly, the proportion of patients who has never had any diagnosis is also higher, resulting in a lower rate of patients receiving medical treatment. Thus, the people in this group do not have a chance to receive preventive care for their complications that might occur after getting ill with the disease. Currently, DM has become the leading causes of morbidity and mortality among Thai people. The hospital admission rate has also risen from 33.3 per 100,000 population in 1985 to 91.0 in 1994 and 586.8 in 2006, as shown in **Figure 1** (Ekachampaka and Wattanamano, 2007).

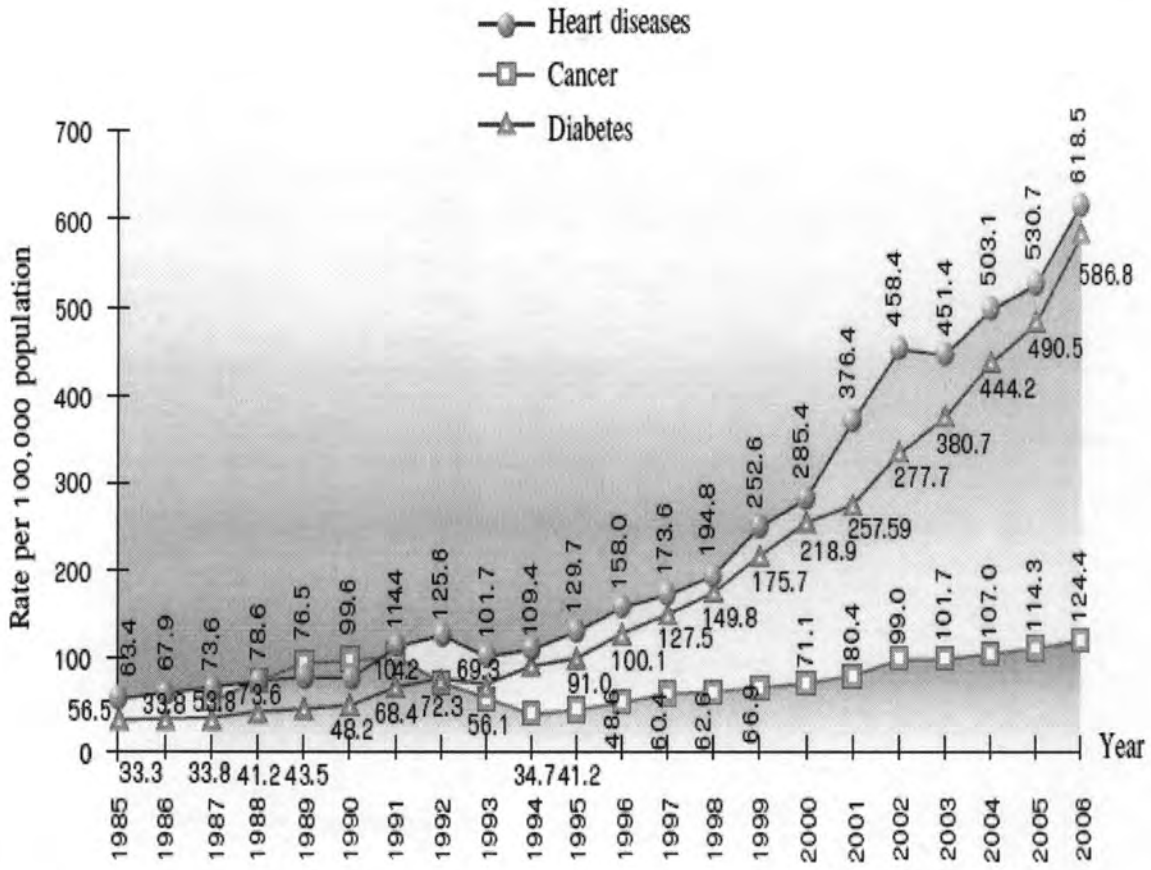

Figure 1 Rate of hospitalizations of patients with heart diseases, cancers or diabetes

Source: Ekachampaka and Wattanamano (2007)

Note: The rate for cancers, since 1994, covers only liver, lung, cervical, and breast cancers

2.5 Treatment of Diabetes Mellitus

The important goals of diabetes treatment are to achieve the best possible control of glycemia, and to reduce the occurrence, progression, and severity of the microvascular and macrovascular complications while minimizing the excess hypoglycemia or other untoward effects (Cook et al., 2007, Isley, 2004). The ADA (2010b) recommended targets for diabetic control for adults that are selected on the basis of practicality and the projected reduction in complications over time, are presented in **Table 2**.

Table 2 Therapeutic goals in patients with diabetes mellitus

Glycemic control	
Fasting plasma glucose	90-130 mg/dl
Hemoglobin A1c (HbA1c)[a]	< 7 %
Prepandrial capillary plasma glucose	70-130 mg/dl (3.9-7.2 mmol/l)
Peak postprandial capillary plasma glucose[b]	< 180 mg/dl (10.0 mmol/l)
Blood pressure	< 130/80 mmHg
Lipid	
Low- density lipoprotein cholesterol (LDL-C)[c]	< 100 mg/dl (< 2.6 mmol/l)
High- density lipoprotein cholesterol (HDL-C)	
Male	> 40 mg/dl (1.0 mmol/l)
Female	> 50 mg/dl (1.3 mmol/l)
Triglyceride	< 150 mg/dl (1.7 mmol/l)

Source: adapted from the American Diabetes Association (2010b)
[a] Referenced to a nondiabetic range of 4.0–6.0% using a DCCT-based assay.
[b] Postprandial glucose measurements should be made 1–2 h after the beginning of the meal, generally peak levels in patients with diabetes.
[c] In individuals with overt CVD, a lower LDL-C goal of 70 mg/dl (1.8 mmol/l)

To achieve the goals, the care of a diabetic individual requires comprehensive diabetes care including diabetes self-management education, assessment of glycemic control, medical nutrition therapy, and optimal medications (AACE Diabetes Mellitus Clinical Practice Guidelines Task Force, 2007; Franz, 2007; Powers, 2008).

2.5.1 Diabetes Self-Management Education

Diabetes self-management education is of utmost importance to ensure understanding, cooperation, and compliance the therapeutic regimen (Isley, 2004). The individuals with DM should receive education about DM, nutrition, physical activity, care of during illness, and medications to lower blood glucose (Powers, 2008).

2.5.2 Assessment of Glycemic Control

Two primary techniques including patient self-monitoring of blood glucose (SMBG) and HbA1c measurement are available for health providers and patients to assess the effectiveness of the management plan on glycemic control. SMBG allows patients to evaluate their individual response to therapy and assess whether glycemic targets are being achieved using blood glucose meter. SMBG is especially important for patients to monitor and prevent asymptomatic hypoglycemia and hyperglycemia.

HbA1c provides a measure of glycemic control over 2-3 months, and has strong predictive value for diabetic complications. Also, HbA1c testing should be performed routinely in all patients with DM, at initial assessment and then as part of continuing care. For any individual patient the frequency of HbA1c testing should be dependent on the clinical situation, the treatment regimen used, and the judgment of the clinician. Conditions that affect erythrocyte turnover (hemolysis, blood loss) and hemoglobin variants must be considered, particularly when the HbA1c result does not correlate with the patient's clinical situation. In addition, HbA1c does not provide a measure of glycemic variability or hypoglycemia (ADA, 2010b; Powers, 2008).

2.5.3 Medical Nutrition Therapy

Medical nutrition therapy (MNT) is the cornerstone of diabetic management throughout all stages of type 2 DM. The goals for MNT for diabetes emphasize the role of lifestyle modifications including nutrition therapy, weight reduction and increasing physical activity in improving glucose control (ADA, 2010b). MNT is an effective monotherapy early in the disease process when there is still reserve insulin secretory capacity. In later stages of type 2 DM, as

pancreatic beta cell function declines, MNT often need to be combined with one or more oral hypoglycemic agents or with insulin for effective glycemic control (O'Connell, 2005). Hypocaloric diets and weight loss (5-7%) often result in rapid dramatic glucose lowering in individuals with new-onset type 2 DM. MNT for type 2 DM should emphasize modest caloric reduction, reduced fat intake, increased physical activity, reduction of hyperlipidemia, and hypertension. Increased consumption of soluble dietary fiber may improve glycemic control in individuals with type 2 DM (Powers, 2008).

1) Nutrition Therapy

Individuals with DM should receive optimal nutrients and energy for body's requirement. The U.S. dietary reference intake (DRI) recommend that adults (in general, not specifically those with DM) should consume 45–65% of total energy from carbohydrate, 20–35% from fat, and 10–35% from protein. The best mix of carbohydrate, protein, and fat appears to vary depending on individual circumstances (Franz, 2003).

Carbohydrate foods which are rich in fiber and have a low energy density are the basis of the eating plan. It is recommended that they contribute up to 65% of the total energy intake. Meals containing carbohydrate are spread evenly through the day. In practice it is recommended that patients with DM have one high fiber, low glycemic index food at each meal. This would include whole grain products, fiber-rich cereals (≥ 5 g per serving), vegetables, and fruits. Other carbohydrate foods (e.g. rice, bakeries, tropical fruits) can be included but in lesser amounts. Sugar alcohols and nonnutritive sweeteners are safe when consumed within the acceptable daily intake levels established by the U.S. Food and Drug

Administration. If adults with DM choose to use alcohol, daily intake should be limited to a moderate amount (≤ 1 drink/day for adult women and ≤ 2 drinks/day for adult men; 1 drink = 15 g alcohol, 360 ml of beer, or 150 ml of wine) (Franz, 2007).

The ADA (2008) recommends that total fat intake should be 25-35% of total calories, and saturated fat less than 7% of total calories. Intake of *trans* fat should be minimized or eliminated. The daily intake of cholesterol should be less than 200 mg/day and 300 mg/day for diabetic patients with and without CVD or dyslipidemia respectively. There is evidence from the general population that foods containing omega-3 unsaturated fatty acids are beneficial, and two or more servings of fish per week can be recommended.

For individuals with DM who has normal renal function, total protein intake should be 10-20% of total calories or 0.8-1.6 g/kg/day. But, for diabetic patients with and renal insufficiency, protein intake should be limited to 0.6 g/kg/day. Dietary protein should be derived from both animal and vegetable sources, however, the source of protein is not especially importance provided that all the essential amino acids are available in adequate amounts. Good-quality protein sources include meat, poultry, fish, eggs, low-fat milk, cheese, and soy. There are few studies with small numbers of subjects with DM suggested that diets with protein contents greater than 20% of total energy may improve blood glucose and insulin concentrations, improve satiety, and reduced appetite (Gannon et al., 2003 and Gannon et al., 2004). However, the effects of protein on regulation of energy intake, satiety, and long-term weight loss have not been adequately studied (Franz, 2007).

Like other aspects of diabetic management, MNT must be adjusted to meet the goals of the individual patient. As for the general population, a diet that

includes fruits, vegetables, fiber-containing foods, low-fat milk is advised. Salt (sodium chloride) intake should be less than 6 g/day for normotensive individuals and less than 3 g/day for hypertensive individuals (Barker, 2002). Currently, evidence does not support supplementation of the diet with vitamins, antioxidants, or micronutrients in diabetic patients who do not have underlying deficiencies (Powers, 2008).

2) Physical Activity

Regular physical activity has been shown to improve glycemic control, improve insulin sensitivity, and reduce cardiovascular risk factors (e.g., hypertension and dyslipidemia) (Franz, 2007). Physical activity is also a primary factor associated with long-term maintenance of weight loss and overall weight control. Initiation of physical activities should begin with a modest increase activity. Walking, swimming and cycling are examples of low-intensity exercises that could be encouraged. Gardening and usual house-cleaning tasks are good exercise as well. Long term goals are to perform at least 150 minutes/week (distributed over at least 3 days) of moderate-intensity aerobic physical activity (50–70% of maximum heart rate). In the absence of contraindications, type 2 diabetic patients should be encouraged to perform resistance training 3 times per week (ADA, 2010b). However, vigorous activity should probably be avoided in the presence of ketosis, hypoglycemia, and untreated proliferative retinopathy (Cook et al., 2007; Powers, 2008).

3) Weight Control

Weight loss is recommended for all overweight and obese individuals with DM. For weight reduction, either low-carbohydrate or low-fat calorie-restricted diets may be effective in the short term (ADA, 2010b). The recommended primary

approach to long-term maintenance of weight loss is therapeutic lifestyle change, which integrates a 500-1,000 kcal/day reduction in calorie intake and an increase in physical activity. To reduce energy intake, sources of hidden energy need to be identified and minimized: for example alcohol, cakes and sweet beverages. A reduction in total energy intake of 500 kcal/day should result in a weight loss of 0.5 kg/week. A slow but progressive weight loss of 0.5-1 kg per week is preferred (Cook et al., 2007).

2.5.4 Medication of Diabetes Mellitus

Patients with DM who are unable to maintain their blood glucose within the normal range by MNT require medication. However, medication should be used in addition to achievement of ideal body weight and lifestyle modification, and not instead of them (Barker, 2002). There are two kinds of medicines including oral hypoglycemic agents and insulin. The selection of the agent to treat patients with DM should be based on effectiveness, safety, tolerability, and cost. Currently, there are six classes of oral hypoglycemic agents including sulfonylureas; biguanides; α-glucosidase inhibitors; thiazolidinediones; glinides; and dipeptidyl peptidase-4 (DPP-4) inhibitors (gliptins). Their mechanisms of action are described in **Table 3** (Maffeo, 2005; AACE Diabetes Mellitus Clinical Practice Guidelines Task Force, 2007).

Table 3 Classes of oral hypoglycemic agents and their mechanisms of action

Drug classes	Generic name	Mechanism of action	Possible adverse effects	Monitoring[a]
1. Sulfonylureas	Glibenclamide Glyburide Glipizide Glimepiride	Stimulates insulin release from pancreatic β-cells	Hypoglycemia Weight gain	FPG at 2 weeks HbA1c at 3 months
2. Biguanides	Metformin	Inhibits hepatic glucose output	Dose-related diarrhea Lactic acidosis in patients with renal compromise	Serum creatinine at initiation FPG at 2 weeks HbA1c at 3 months
3. Alpha-glucosidase inhibitors	Acarbose Miglitol	Delays carbohydrate absorption to decrease postprandial hyperglycemia	Dose-related diarrhea, abdominal pain, flatulence	PPG at initiation HbA1c at 3 months
4. Thiazolidinediones	Pioglitazone Rosiglitazone	Enhances insulin sensitivity	Edema, weight gain, and congestive heart failure	AST and ALT at baseline Monitor for signs of fluid overload
5. Glinides	Repaglinide Nateglinide	Stimulates insulin secretion	Hypoglycemia	PPG at initiation FPG at 2 weeks HbA1c at 3 months
6. Gliptins	Sitagliptin	Inhibits dipeptidyl peptidase-4 activity Restores GLP-1 and GIP levels	Not clinically significant	PPG at initiation FPG at 2 weeks HbA1c at 3 months

Source: AACE Diabetes Mellitus Clinical Practice Guidelines Task Force, 2007
Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; GIP = glucose-dependent insulinotropic polypeptide; GLP-1 = glucagons-like peptide 1; FPG = fasting plasma glucose; HbA1c = hemoglobin A1c; PPG = postprandial glucose
[a]All measurements should be performed at the time noted after initiation of therapy and thereafter as directed by the patient's physician

In patients with newly diagnosed type 2 DM in whom insulin therapy is not indicated pharmacologic therapy with either a sulfonylureas or metformin be initiated as monotherapy, as long as no contraindications are present. When used as monotherapy, sulfonylureas and metformin are equally effective in decreasing blood glucose level. Because metformin promotes weight loss and reduces lipid level, it is preferred in overweight patients with type 2 DM and dyslipidemia. In lean type 2 diabetic patients, therapy with either a sulfonylureas or metformin can be initiated. If monotherapy with sulfonylureas or metformin fails to achieve the desired level of glycemic control, a second oral agent should be added. If combination therapy with two oral agents does not achieve the desired goal, a third oral hypoglycemic agent or insulin might be added (DeFronzo, 1999; Suwittayarat, 2006).

2.6 Insulin Resistance and Assessment of Insulin Resistance

Insulin resistance has been broadly defined as a state of a cell, tissue, or organism in which a greater than normal amount of insulin is required to elicit a quantitatively normal response (Mantzoros and Flier, 1995). Simply stated, insulin resistance is a reduced physiological response of the body cells to the action of insulin. Insulin resistance is the main cause for the development of type 2 DM. Moreover, it is also an underlying cause of metabolic syndrome, which, if neglected, may lead to CVD and stroke (Franz, 2007; Hanley et al., 2002).

Assessment of insulin resistance is important in order to understand the aetiopathology of type 2 DM, to examine the epidemiology and to assess the effects of intervention. Currently, clinical assessment of insulin resistance (or, conversely, insulin sensitivity) relies on several tests include determination of insulin levels, either

at baseline (fasting) or after OGTT, assessment of sequential plasma glucose levels after the intravenous administration of insulin (ITT), estimation of an index of insulin sensitivity by applying minimal-model analysis (MINMOD) of frequently sampled insulin levels during an intravenous glucose tolerance test, and the measurement of *in vivo* insulin-mediated glucose disposal by the euglycemic hyperinsulinemic clamp procedure (Tritos and Mantzoros, 1998). The gold standards for measuring insulin sensitivity are the euglycemic hyperinsulinemic clamp and MINMOD but these methods are time-consuming, invasive, expensive, and technically difficult to apply in a clinical setting or for large populations. For this reason, simpler, less-invasive techniques of determining insulin resistance, based on measuring fasting blood insulin and glucose, have been developed. The homeostasis model for insulin resistance and the quantitative insulin sensitivity check index (QUICKI) are the most commonly used surrogate measures and provide a reliable alternative to the glucose clamp (McAuley et al., 2001; Lee et al., 2006).

Homeostasis model assessment (HOMA) is a method for assessing β-cell function and insulin resistance from fasting blood glucose and insulin or C-peptide concentrations. The HOMA has been shown to be a reliable estimate of insulin resistance both among individuals with and without type 2 DM (Bonora et al., 2000). This method can be used to assess longitudinal changes in insulin resistance and β-cell function in patient with DM in order to examine the natural history of DM and to assess the effects of treatment. The HOMA of insulin resistance (HOMA-IR) and β-cell function (HOMA-B%) were index of insulin resistance and β-cell function respectively. The HOMA models were calculated from fasting blood insulin (μIU/ml) and glucose (mmol/l) values with the following formulas (Matthews et al., 1985):

$$\text{HOMA-IR} = \frac{\text{fasting blood insulin} \times \text{fasting blood glucose}}{22.5}$$

$$\text{HOMA-B\%} = \frac{20 \times \text{fasting blood insulin}}{\text{fasting blood glucose} - 3.5}$$

The HOMA–IR and HOMA-B% value correlates well with clamp techniques (r^2 = 0.58-0.88 and r^2 = 0.62-0.69 respectively) and has been frequently used to assess changes in insulin sensitivity after treatment. Reciprocal index of HOMA (1/HOMA-IR) can be used to assess insulin sensitivity (Wallace et al., 2004).

2.7 Whey Protein

Whey protein is one of the two classes of milk protein in which is found in whey. Whey is a co-product of cheese-making and casein manufacture in the dairy industry. After the casein curd separates from the milk, following coagulation of the casein proteins through the action of rennet or mineral/organic acid, the remaining liquid is called whey (Smithers, 2008). Whey can be made from any type of milk, with bovine milk being the most popular. Bovine milk contains about 3.4-3.6% protein by weight (**Figure 2**). Of the total protein about 80% is casein and 20% is whey protein (Miller et al., 2006). All whey products for human use must be pasteurized. Pasteurized whey is processed to provide a wide range of products, including condensed whey, dry whey, and modified whey products, each with unique functional characteristics.

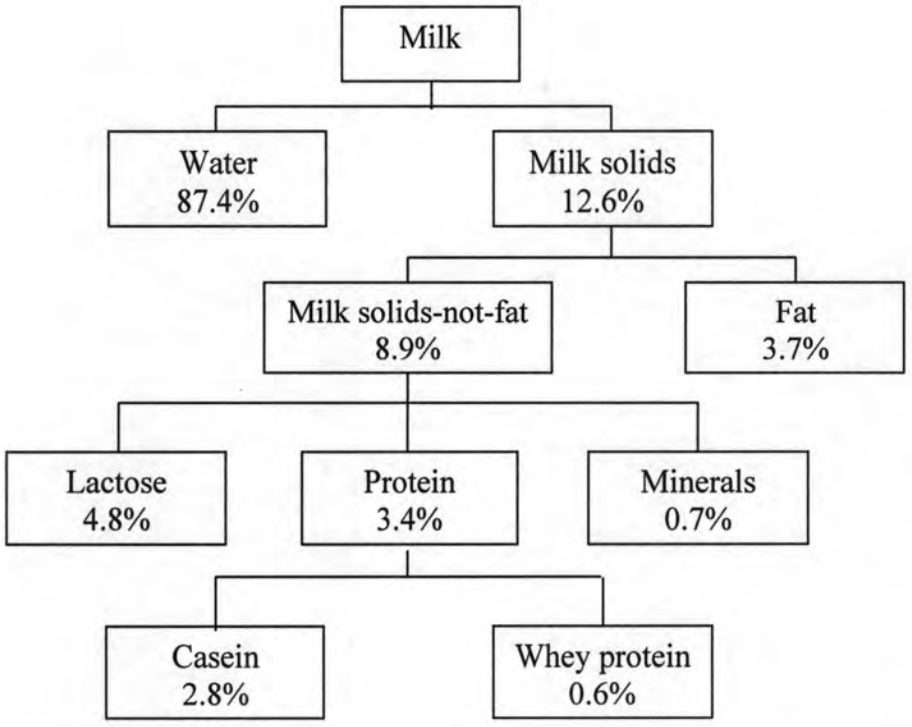

Figure 2 Major constituents of bovine milk

Modified whey products may be obtained by various processes, such as reverse osmosis, microfiltration, ultrafiltration, dialfiltration and ion exchange. Examples of modified whey products include delactosed whey, whey protein concentrate (WPC), whey protein isolate (WPI), and whey protein hydrolysate (WPH). These whey products are easily transported, have enhanced storage stability, blend well with other foods, and are economical sources of milk solids. Three products of whey protein that are commonly used in clinical studies are described (Miller et al., 2006; Walstra, 2006; U.S. Dairy Export Council, 2004):

Whey protein concentrate (WPC) contain 20-89% protein on a solids basis and little, if any, lactose or fat. Traditionally, WPC is made of delactosed, demineralized whey. The process starts by concentrating whey about tenfold by evaporation, to obtain lactose crystals. The mother liquid then is demineralized.

Drying of the remaining liquid results in WPC. A considerable proportion of the protein is more than 25%. To obtain a higher protein concentration ultrafiltration and diafiltration were generally used. The liquid is spray-dried and resulting WPC powder can be highly soluble. WPC, the most common and affordable form of whey, is used in protein beverages and bars, bakery and confectionary products, dairy foods and other nutritional food products.

Whey protein isolate (WPI) contains at least 90% protein on a solids basis and little, if any, lactose or fat. WPI can be produced, like WPC, by ultrafiltration; at least one diafiltration step then is necessary. Moreover, WPI is produced by ion exchange. Processing of WPI is shown in **Figure 3**. This form of whey is commonly use as an ingredient in infant formulas, sport nutrition products, protein supplement products, protein beverages and bars and other nutritional food products.

Whey protein hydrolysate (WPH) is obtained by hydrolysis of whey protein. WPH are manufactured by acid or enzyme digesting, at controlled temperature and pH, and then filtering and spray drying the resulting solution. WPH uses in high value specialist nutritional applications such as tube-feeding preparations, infant formulas, sport nutrition, medical nutrition and special dietary supplements. WPH is the highest digestible form containing easy-to-digest peptides. Appropriately hydrolyzed proteins lose the ability to induce allergic reactions in susceptible people, and so can be used in hypoallergenic infant formulas. However, a problem is that some peptides can be bitter on the tongue.

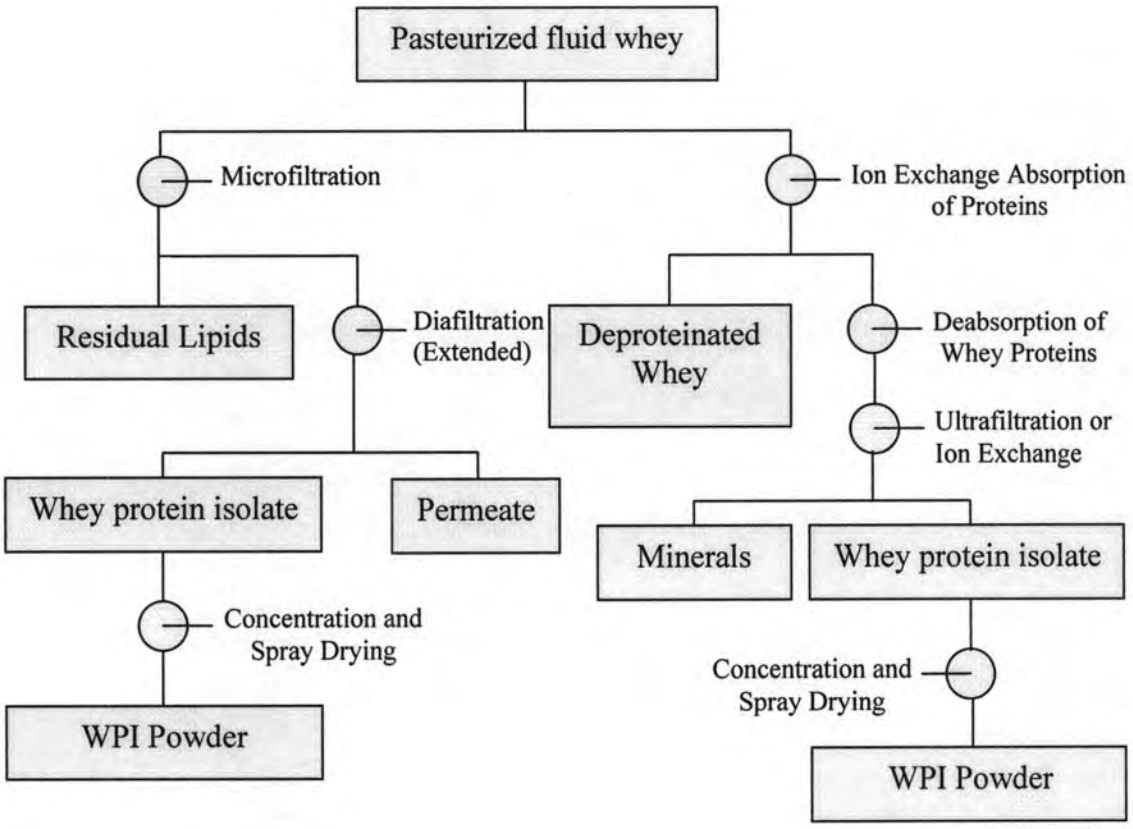

Figure 3 Processing of whey protein isolate

2.7.1 Composition of Whey Protein

Whey protein consists of several different proteins, including beta-lactoglobulin, alpha-lactalbumin, immunoglobulins, bovine serum albumin, lactoferrin, lactoperoxidase, glycomacropeptides, and together with other minor components. Each of which has specific biological and functional properties. The composition of whey protein and their main biological functions are summarized in **Table 4**.

Table 4 Composition of whey protein

Whey Components	Percent of whey protein	Benefits
Beta-lactoglobulin	50-55	Source of EAAs and BCAAs
Alpha-lactalbumin	20-25	Primary protein found in human breast milk. Source of EAAs and BCAAs
Immunoglobulins	10-15	Primary protein found in colostrum Immune modulating benefits
Glycomacropeptides	10-15	Source of BCAAs Lacks the aromatic amino acids (phenylalanine, tryptophan, and tyrosine)
Bovine serum albumin	5-10	Source of EAAs
Lactoferrin	1-2	Antioxidant Antibacterial, antiviral , and antifungal Promotes growth of beneficial bacteria Naturally occurs in breast milk, tears, saliva, bile, blood, and mucus
Lactoperoxidase	0.25-0.5	Inhibits growth of bacteria

EAAs = essential amino acids; BCAAs = branched-chain amino acids

Source: adapted from Marshall, 2004

1) Beta-lactoglobulin (β-LG)

β-LG is the most prevalent protein in whey protein, represents about 50% of the total whey protein content, while human milk contains no β-LG. β-LG has numerous binding sites for minerals, fat soluble vitamins and lipids, and acts as a transport protein for desirable lipophilic compounds such as tocopherol and vitamin A.Thus, β-LG has the potential to modulate lymphatic responses (Perez and Calvo, 1995; Marshall, 2004). The amino acid content of β-LG is rather important, because besides being a source of EAAs and BCAAs, it is a source rich in cysteine, which is important for synthesis of glutathione (Madureira et al., 2008).

2) Alpha-lactalbumin (α-lactalbumin)

Alpha-lactalbumin is one of the main proteins found in human and bovine milk. This protein comprises approximately 20-25% of bovine whey protein. It possesses an excellent amino acid profile which is rich in lysine, leucine, threonine, tryptophan and cystine. Purified α-lactalbumin has the most structurally similar protein profile compared to human milk. Therefore, this protein is most used in infant formula to make it more similar to human milk (Yadav and Brew, 1991; Marshall, 2004). This whey protein has been demonstrated to have immune enhancing properties and may be effective as an anti-cancer agent in several types of cancer (Medureira et al., 2007).

3) Immunoglobulins (Ig)

Immunoglobulins comprise approximately 10-15% of the total whey protein content. Immunoglobulins are a complex group of proteins that make a significant contribution to the protein content as well as exerting an important immunological function. There are four classes of immunoglobulins in bovine milk including IgG1, IgG2, IgA and IgM, while human antibodies include IgA, IgD, IgE, IgG, and IgM. IgG constitutes approximately 75% of the antibodies in an adult. There is evidence that IgG may have a role in disease control in adults. Moreover, IgG is well recognized to provide disease protection to newborns through passive immunity. IgG is transferred from mother to child in utero via cord blood and by breast-feeding, and serves as a child's first line of immune defense. (Kilara and Vaghela, 2004; Marshall, 2004).

4) Glycomacropeptide (GMP)

GMP is also referred to as casein macropeptide. GMP is a protein present in whey at 10-15%, due to the action of chymosin on casein during the cheese-making process. This protein is high in branched chain amino acids and lacks the aromatic amino acids (phenylalanine, tryptophan, and tyrosine). It is one of the few naturally occurring proteins that lacks phenylalanine, making it safe for individuals with phenylketonuria (Marshall, 2004).

5) Bovine serum albumin (BSA)

BSA is a large protein that makes up approximately 5-10% of total whey protein. BSA is a source of essential amino acids, but there is very little available information regarding its potential therapeutic activity.

6) Lactoferrin

Lactoferin is an iron-binding glycoprotein, is a non-enzymatic antioxidant found in the whey fraction of milk as well as in colostrums. The concentration of lactoferrin in most commercial whey protein powders is only 0.35-2.0% of total proteins. This protein is present in the human body as a secretor protein. It is synthesized by glandular epithelial cells and mature neutrophils. This protein is found in milk, saliva, tears, nasal and intestinal secretions, pancreatic juice, as well as in secondary granules of neutrophils (Lonnerdal and Iyer, 1995). Lactoferrin appears to play several biological roles. It is appeared to have antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant and immunomodulatory activities. Moreover, it promotes the growth of beneficial intestinal bacteria such as *Lactobacillus bifidus*, helping infants establish good microbial conditions in the

intestines. In some countries, this protein is added to infant formulas to make them more similar in protein composition to human milk (Marshall, 2004).

7) Lactoperoxidase (LP)

LP is the most abundant enzyme found in whey. It accounts for 0.25-0.5% of total protein found in whey. The majority of LP ends up in whey following the curding process. It has the ability to catalyze certain molecules, including the reduction of hydrogen peroxide. This enzyme system catalyzes peroxidation of thiocyanate and some halides (such as iodine and bromium), which ultimately generates products that inhibit and/or kill a range of bacterial species. During the pasteurization process, lactoperoxidase is not inactivated, suggesting its stability as a preservative (Marshall, 2004).

2.7.2 Nutritional Value of Whey Protein

The proteins in whey are easily digested, absorbed and have excellent metabolic efficacy, giving the protein a high nutritional quality. The nutritional quality of a protein can be expressed in various ways. Protein Efficiency Ratio (PER), Protein Digestibility (PD), Biological Value (BV), Net Protein Utilization (NPU) and Protein Digestibility Corrected Amino Acid Score (PDCAAS) are frequently used to indicate the potency of a food protein as a source of amino acids. By all measures, whey proteins offer excellent protein quality (**Table 5 and Table 6**) (Pasin and Miller, 2004; Walzem, 2004).

Table 5 Protein quality comparison of whey protein and other key proteins

Protein Source	Protein quality		
	BV	PER	NPU
Whey Protein Concentrate	104	3.2	92
Soy protein	74	2.1	61
Whole egg	100	3.8	94
Bovine milk	91	3.1	82
Casein	77	2.5	76
Beef	80	2.9	73

BV = Biological Value; PER = Protein Efficiency Ratio; NPU = Net Protein Utilization
Source: Pasin and Miller, 2004

Table 5 Protein digestibility corrected amino acid score of whey protein and other key proteins

Protein Source	Protein Digestibility Corrected Amino Acid Score (PDCAAS)
Whey protein isolate	1.14
Casein	1.00
Milk protein isolate	1.00
Soy protein isolate	1.00
Egg white powder	1.00
Ground beef	1.00
Wheat gluten	0.25

Source: Pasin and Miller, 2004

Whey protein is a good source of the essential amino acids (EAAs) when compared with other typical food proteins. Whey protein contains high amount of branched-chain amino acids (BCAAs) including leucine, isoleucine, and valine (about 20-26 grams per 100 grams of protein). These amino acids have unique roles in human metabolism; in addition to provide substrates for protein synthesis, suppress protein catabolism and serve as substrates for gluconeogenesis (U.S. Dairy Export Council, 2004; Smithers, 2008). Leucine in particular triggers muscle protein synthesis which is sensed by the insulin-signaling pathway. Leucine is an important factor in tissue growth and repair (Haug et al., 2007). Additionally, whey protein contains high amounts of amino acids arginine and lysine, which serve a biological role as a stimulator of muscle growth. For this reason, BCAAs help minimize muscle wasting under conditions of increase protein breakdown, which makes whey protein beneficial for athletes, body builders and individuals with such disease as HIV and cancer (Balch, 2006; Miller et al., 2006).

Whey protein is a rich and balanced source of the sulfur-containing amino acids cysteine and methionine. These amino acids serve a critical role as antioxidants, as precursors to the potent intracellular anti-oxidant glutathione, and in one-carbon metabolism. This suggests that their ingestion may contribute to increase the level of free cysteine, consequent production of gluthatione, and immune function is enhanced through glutathione function (Marshall, 2004; Madureira et al., 2007; Smithers, 2008).

2.7.3 Functional Properties and Applications

The uses of whey protein products for human consumption have greatly expanded as a result of recognition of excellent nutritional and functional properties of whey protein. Whey protein is widely used in various products such as infant formulas, dietary supplements, medical nutrition, sport bars and beverages (Miller et al., 2006). Whey proteins are used in many food applications because of its functionality and nutritive value. Whey proteins possess solubility over a wide pH range, create viscosity through water-binding, form gels, emulsify, bind fat, facilitate whipping, foaming and aeration, enhance color, flavor and texture, and offer numerous nutritional advantages. For this reason, they can modify some or all of the organoleptic, visual, textural and rheological properties of food, resulting in improved consumer acceptance of the food products (Borrington, 2004; Tunick, 2008). Some applications of whey proteins are showed in **Table 7**.

Table 7 Functional benefits of whey products

Function	Benefits	Applications
Adhesion	Help adhere bread crumbs or batters to meat, fish or vegetables; seeds to bread products; and glazes to bakery products	Breads, breaded products, baked goods
Antioxidant activity	Prevent lipid oxidation in pre-cooked meats such as pork and salmon	Pre-cooked meats
Browning	Contribute to browning in baked or microwaved products, or in caramel confections	Baked goods, confections
Dispersibility	Dissolve quickly in water without excessive agitation	Dry beverage mixes
Emulsification	Creates stable emulsions and prevents fat globules from forming one large mass	Baked goods, beverages, meat and seafood products, ice cream mixes, mayonnaise-type dressings
Flavor Enhancement	Bring out already present flavors, or add flavors of their own	Baked goods, beverages, confections, dairy products processed meats, snacks
Gelling and heat setting	Maintain moistness, add opacity, improve texture and mouthfeel	Baked goods, beverages, meat, seafood products, and dairy products such as
Neutrality	Clean flavor, no off flavors in finished product	Confections, frozen desserts
Nutritional enrichment	Increase nutritional content of products Contribute to a food's healthful image and clean label	Baked goods, beverages, dairy products, infant formula, soups, sauces, meat and seafood products
Solubility	Easily dispersed in most food systems. Prevents sedimentation in beverages, soups and sauces	Bakery, beverages, soups, sauces, confections, frozen desserts, yogurt, and infant formula
Water binding and viscosity building	Provide fat-like attributes in products, allowing a reduction in fat content. Improve product texture, creating moister products	Baked goods, beverages, dairy products, creamers, soups, sauces, chopped meat and seafood products
Whipping, foaming and aeration	Maintain foam properties, which enhances visual appeal of the finished product, as well as taste and texture	Baked products such as meringues and cakes, confections, frozen desserts

Source: U.S. Dairy Export Council, 2008

2.7.4 Health Benefits of Whey Protein

The health benefits of whey protein are being documented through a series of studies including in vitro, animal, and human, which show use of whey protein for clinical indications such as cancer, hepatitis, human immunodeficiency virus (HIV) disease, and obesity (Onwulata, 2008). Several studies reported that whey protein could reduce postprandial glucose, cholesterol, body weight, blood pressure, increase postprandial insulin secretion and insulin sensitivity that may decrease the risk of or severity of type 2 DM and CVD. Moreover, whey protein may confer several other health benefits including immunomodulatory, antimicrobial, antiviral, anticancer, and psychomodulatory, as well as gut and bone health enhancing properties (Marshall, 2004; Yalcin, 2006; Morris and FitzGerald, 2008; Smithers, 2008). This section review intends to focus on scientific evidence related to the biological activities of whey protein on blood glucose level, lipid profile, insulin resistance, body weight, blood pressure.

According to study of Belobrajdic et al. (2004), insulin-resistance Wistar rats were separated randomly into 4 dietary treatment groups (n = 8/group) and fed test diets for 6 weeks. The low-protein diets contained WPC or red meat (80 g /kg diet), and the high-protein diets WPC or red meat (320 g/kg diet). The results showed that a high-protein diet reduced energy intake and visceral, subcutaneous, and carcass fat in mature rats by up to 26% compared to a low-protein diet. Dietary WPC also reduced fasting blood insulin concentration by 40% ($p < 0.05$) and increased insulin sensitivity, compared to red meat ($p < 0.05$). Thus, these findings support that a high-protein diet reduces energy intake and adiposity and

that whey protein is effective in reducing body weight and increasing insulin sensitivity.

Janle and Lachcik (2008) conducted a study to test the hypothesis that a high whey protein diet would be the most beneficial for type 2DM. Zucker diabetic rats were fed with a high diet containing either content of whey protein or soy protein for 8 weeks. Results showed that whey-fed rats had a trend toward lower glucose levels in interstitial fluid (ISF), and a trend toward lower blood glucose levels over 3 hours compared to rats fed soy protein. ISF over 3 hours was significantly lower in the two groups. There were no significant differences in insulin concentrations, insulin sensitivity, food consumption, and weight gain between groups.

A number of human studies reported that whey protein exhibited stimulating effect on postprandial insulin secretion in both healthy and type 2 diabetic subjects. Calbet and MacLean (2002) reported a two to fourfold increase in insulin secretion in six healthy subjects following administration of a whey protein hydrolysate (0.25 g/kg body weight) after 30 minutes compared to the response obtained with a glucose solution (25 g/L) and bovine milk. The insulin response was correlated to the increase in plasma levels of leucine, isoleucine, valine, phenylalanine, and arginine. Nilsson et al. (2004) investigated the effect of dietary sources of proteins on levels of postprandial blood glucose, insulin, amino acids, and incretin hormones (glucose-dependent insulinotropic polypeptide, GIP; glucagon-like peptide 1, GLP-1). Twelve healthy volunteers were served test meals consisting of reconstituted milk, cheese, whey, cod, and wheat gluten with equivalent amounts of lactose. An equi-carbohydrate load of white-wheat bread was used as a reference meal. Results indicated that reconstituted milk powder and whey had substantially

lower area under the curve (AUC) for postprandial glucose than did the bread reference (-62% and -57% respectively). Whey meal was accompanied by higher AUC for insulin (90%) and GIP (54%). A positive correlation was obtained between responses of insulin and GIP concentrations. Therefore, the study suggested that milk proteins have insulinotropic properties and whey fraction contains the predominating insulin secretagogue.

A similar study was conducted to assess effects of supplementation of meals having a high glycemic index with whey protein on blood insulin and glucose response in type 2 diabetic subjects (n = 14). Whey powder (27.06 g) was supplemented at breakfast and lunch on day 1 and was exchanged for lean ham (96 g) and lactose (5.3 g) on day 2. Results showed that the levels of insulin and GIP were significant higher when meals were supplemented with whey protein (Frid et al., 2005).

It has been postulated that the postprandial blood insulin increasing is a result of elevated concentrations of specific insulinogenic amino acids as well as bioactive peptides, either originally present in whey or formed during digestion. Another possibility is the effect of whey protein on incretin hormones. Incretin hormones such as GLP-1 and GIP are released from the gut after food consumption and are involved in many digestive roles including glycemic control (Frid et al., 2005; Petersen et al., 2009).

Besides insulinotropic property of whey protein, several animal and human studies reported that whey protein intake could improve lipid profile, decrease body weight, fat mass, and blood pressure. These may decrease the risk of or severity of CVD. In animal models, Zhang and Beynen (1993) studied the effect of dietary

whey protein versus casein on plasma and liver cholesterol concentrations in female-weanling Wistar rats. The rats were divided into 8 groups (n = 12/group) to received the high-cholesterol diets (10 g cholesterol/kg feed) containing either 150 or 300 g of whey protein, casein, or the amino acid mixtures. The diets were fed for 3 weeks. The results showed that at the low dietary protein level, whey protein versus casein did not affect blood total cholesterol, but lowered the concentration of liver cholesterol. At the high dietary protein level, whey protein significantly lowered blood and liver cholesterol and also blood triglyceride. At the high dietary protein concentration, whey protein reduced the faecal excretion of bile acids when compared with casein. The researchers suggested tentatively that the cholesterol-lowering effect of whey protein in rats is caused by inhibition of hepatic cholesterol synthesis.

Consistently, Beena and Prasad (1997) investigated the cholesterol lowering properties of milk and fermented milk products in albino rats. The rats were fed with a basal diet, basal diet plus cholesterol, and basal diet plus cholesterol together with whole milk or standard or bifidus yogurt for 30 days. The yogurts were fortified with skim milk powder, condensed whey or lactose-hydrolyzed condensed whey. They found that standard yogurt containing lactose-hydrolyzed condensed whey and all bifidus yogurts lowered serum cholesterol, but whole milk and ordinary yogurt had no cholesterol lowering effect. There was marked lowering of LDL-C in rats given either type of yogurt fortified with whey proteins. This study had demonstrated that bifidus yogurts and yogurts fortified with whey protein could reduce total-C and LDL-C.

Whey proteins modulate several hormones that influence weight and body composition. Kasim-Karakas et al. (2007) conducted a study to compare the

effects of acute protein administration with those of glucose challenges on hormones related to obesity and insulin resistance (cortisol and insulin) and hunger (ghrelin). Overweight and obese women with polycystic ovary syndrome (n = 28) were tested with a 5-h oral-glucose-tolerance test (OGTT) and a euvolemic, euenergetic protein challenge (75 g of WPI per dose). Results indicated that WPI ingestion caused significantly lower fluctuations in blood glucose, lower hyperinsulinemia (less lipogenesis), and lower cortisol levels (lean muscle preservation). Furthermore, WPI intake suppressed ghrelin significantly longer than did glucose, which suggested a prolonged satietogenic effect.

Baer et al. (2006) conducted a double-blind, randomized clinical trial to determine the effects of supplemental whey protein, compared to soy protein and an isocaloric amount of carbohydrate, on body weight and composition in free-living, overweight and obese subjects. Ninety subjects were randomized for 6 months to one of three treatment groups: 1) 60 g/d of whey protein, 2) 60 g/d of soy protein or 3) a control group receiving 60 g/d of carbohydrate. After 6 months, body weight of the group consuming the whey protein was 1.8 ± 0.6 kg (2%) lower than the group consuming the carbohydrate treatment ($p < 0.006$). However, body weight was not different between the groups consuming the soy protein and whey protein or between the groups consuming the soy protein and carbohydrate treatment. Body fat (measured by body air-displacement plethysmography) was 2.3 ± 0.8 kg lower in the whey protein group compared to the carbohydrate group ($p < 0.005$). Lean body mass was not different among groups. Waist circumference was lower ($p < 0.001$) in the whey protein group than the two other groups. The change in body weight is associated with a decrease in body fat without affecting lean body mass.

The same results were found in a randomized, double-blind, parallel-arm, 12-week study of Frestedt et al. (2008). Obese men and women subjects (BMI of 30–42 kg/m^2) were counseled to reduce caloric intake by 500 kcal/day and consumed either 20 g daily of WPI (treatment) or an isocaloric beverage (control) 20 minutes before breakfast and 20 minutes before dinner. At the end of the study, significant reduction in body weight was observed in both groups (3.82 ± 0.55 kg for WPI group and 3.24 ± 0.47 kg), but the amount of weight loss was not significantly different between groups. Treatment group also lost significantly more body fat compared to control group. Keogh and Clifton (2008) investigated effect of meal replacements high in glycomacropeptide (GMP) on weight loss and markers of cardiovascular risk in 127 overweight and obese subjects (BMI > 27 and < 40 kg/m^2). Meal replacements contained 15 g protein from GMP-enriched WPI (GMP-WPI) or skim milk powder (SMP). Subjects consumed 2 sachets daily instead of 2 meals for 6 months and 1 sachet daily for a further 6 months. Results indicated that weight loss at 6 months was 10.3 ± 5.8 and 11.0 ± 5.8 kg, and at 12 months was 9.9 ± 8.8 and 10.8 ± 7.4 kg, for the GMP-WPI and SMP groups respectively ($p < 0.001$, compared with baseline). At months 6 and 12, total-C, LDL-C, TG, glucose, insulin, SBP and DBP decreased significantly from baseline in both groups, while HDL-C increased at month 12.

Focusing on effect of whey protein on blood pressure, hypotensive effect of whey protein and its bioactive peptides were reported in several studies. Studies in spontaneously hypertensive rats demonstrated that potent angiotensin converting enzyme (ACE) inhibitory peptides hydrolyzed from whey proteins can reduce blood pressure from 2-33 mmHg (Yamamoto et al. 1999, FitzGerald et al., 2004). A limited number of human studies demonstrated that whey protein and

fermented dairy products, which have been shown in vitro to contain ACE- inhibitory peptides, can decrease blood pressure (Aihara et al., 2005; Kawase et al., 2000; Keogh and Clifton, 2008; Hata et al., 1996; Pins and Keenan, 2002; Seppo et al., 2003).

Kawase et al. (2000) studied effect of administration of fermented milk containing WPC to rats and healthy men on serum lipids and blood pressure. The results showed that serum total-C level for the group fed fermented milk with both *Lactobacillus casei* TMC0409 and *Streptococcus thermophilus* TMC 1543 was significantly lower than that of the control group. Furthermore, the effect of this fermented milk on the serum lipid level in twenty healthy adult men was investigated. During the 8-week study, the volunteers consumed 200 ml of fermented milk or placebo in the morning and evening. After 8 weeks, SBP lowered significantly by the intake of fermented milk while such effect was not observed in the placebo group. HDL-C level showed a significant rise and TG level lowered significantly in the fermented milk group. The atherogenic index [(non-HDL cholesterol)/HDL-C] for the fermented milk group decreased significantly from 4.34 to 3.52 ($p < 0.05$).

Pins and Keenan (2002) studied the effect of hydrolyzed WPI in mild hypertensive subjects (BP $\geq$ 120/80 mmHg and $\leq$ 155/95 mmHg) who were not taking any medications. The subjects (n = 30) were randomized to receive 20 g daily of either hydrolyzed WPI (treatment) or intact WPI (control) for 6 weeks. After completion of treatment, there was a mean reduction of 8.0 $\pm$ 3.2 mmHg in SBP ($p < 0.05$) and of 5.5 $\pm$ 2.1 mmHg in DBP ($p < 0.05$) in the treatment group compared with the control group. In addition to lowering of blood pressure, ACE activity decreased 25% and bradykinin increased 2.46-fold. The high-sensitivity

C-reactive protein and LDL-C were significantly improved by both treatments. Investigators suggested that whey-derived peptides might be available treatment option for pre-hypertensive and/or stage-1 hypertensive individuals. Consistently, 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 (n = 70). They found that SBP in the whey and casein groups decreased significantly at weeks 6 and 12, compared to baseline. At week 12, DBP in the whey and casein groups decreased significantly compared to baseline and those of the control group. This study concluded that supplementation with whey protein improves blood pressure and vascular function in overweight and obese individuals. Additionally, result from a meta-analysis of 15 placebo-controlled clinical trials on effect of peptides derived from food proteins (fermented milk, whey protein, casein, fish) on blood pressure showed that peptides derived from food proteins can significantly reduce SBP and DBP, with pooled mean effects of -5.13 mmHg and -2.42 mmHg respectively (Pripp, 2008).

2.7.5 Adverse Effects of Whey Protein

As a component of milk, whey has a long history of human use. It is a natural food substance and is as safe to use as milk. Commercial whey protein products are considered a generally recognized as safe (GRAS) substance for food product applications (U.S. Food and Drug Administration, 2008). Currently, there is not enough documented information about whey protein adverse effects. According to clinical studies, whey protein is generally well tolerated and safe to consume. No serious adverse reactions have been noted following administration of whey

protein (Bounous et al., 1993; Frestedt et al., 2008. Grey et al., 2003; Kasim-Karakas et al., 2009; Kennedy et al., 1995; Keogh and Clifton, 2008; Micke et al., 2002; Pins and Keenan, 2002; Tienboon et al., 2009). However, minor adverse effects of whey protein including gastrointestinal disturbance, bloating, and flatulence were reported. The adverse effects were most troublesome on initiation of treatment although often improved after a few days (Micke et al., 2001 and 2002; Tienboon et al., 2009). Because whey protein is derived from milk, people who are allergic to dairy products or lactose intolerant should consult their physicians before supplementing whey protein. Recent advances in purification techniques have afforded lactose-free whey products which would reduce the risk of adverse reactions for these individuals (Marshall, 2004).