CHAPTER 1

Introduction

1.1 Statement and significance of problem

Obesity nowadays becomes a growing concern worldwide. Thailand is the top five Asia-pacific countries with the highest percentage of obese people. The number of obese people of Thailand in 2015 will have approximately 21 million according to the Ministry of Public Health. Obesity is a highly serious health problem because of its relation to various diseases including hypertension, cardiovascular disease, cancer and especially type II diabetes.

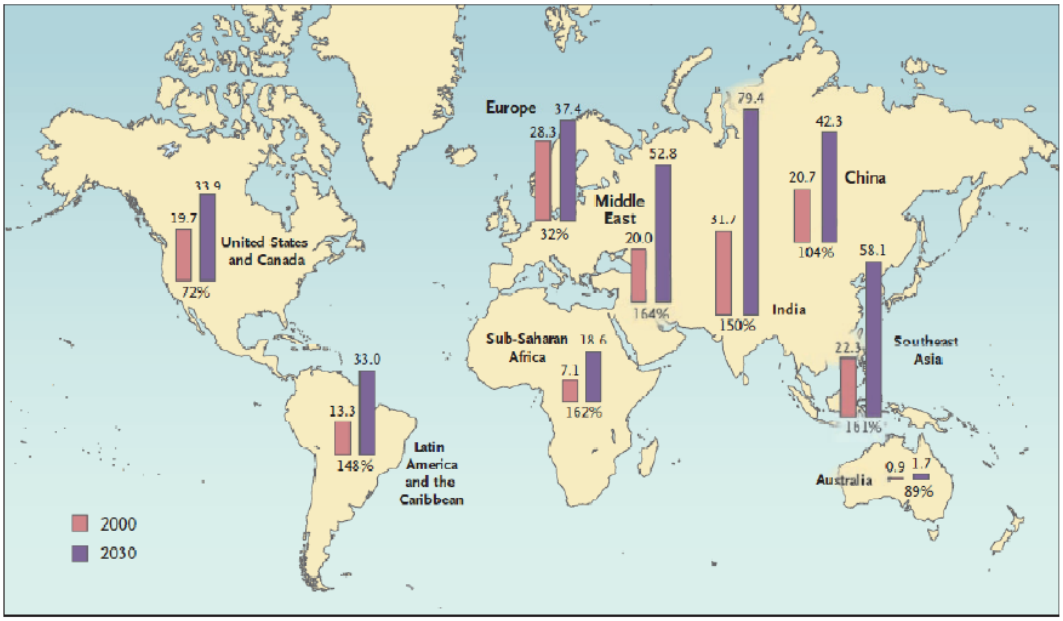
Obesity is a condition characterized by increasing adipose tissue mass that cause from increased fat cell number (hyperplasia) or/and size (hypertrophy). Hyperplasia leads to the incorrigible expansion of adipose tissue in unwanted depots, specifically the visceral cavity. Hypertrophy leads to the dysfunction of adipocytes, as they become insulin resistance and recruit macrophages to the tissue for their own destruction. Together, these events promote a chronic inflammation in adipocytes and lead to the metabolic syndrome development. Therefore, identification and targeting potential factors that regulate obesity-induced inflammation and insulin resistance in adipocytes are of great importance in the prevention and treatment of obesity and diabetes.

Rice has always been the vital food of the Thai people as it plays a precious role in the essence of Thai life. Health profits of rice include giving fast and immediate energy sources, stabilizing blood sugar levels, helping bowel movement and providing essential source of vitamin to human body. Rice varies in texture, size, shape, aroma and stickiness. It is also available in a number of colors, including white, brown, red and purple. Purple rice (*Oryza sativa* L.) is a special variety of rice with a unique characteristic purple pigment in their pericarp and husks (hull). The major component in

the purple pigment is anthocyanin. Anthocyanin has various excellent biological activities including antioxidant, anti-inflammation, anti-cancer, and specifically hypoglycemic. Therefore, purple rice diet may improve prevention and/or treatment of the diseases. To further extend the scientific knowledge for promotion of the purple rice consumption, this study aims to determine the effect of purple rice on adipogenesis and inflammation-induced insulin resistance in adipocytes.

1.2 Literature reviews

1.2.1 Obesity and adipocyte



Millions of Cases of Diabetes in 2000 and Projections for 2030, with Projected Percent Changes.

Figure 1.1 Prospective development of diabetes in the future worldwide (1).

Obesity is a leading avoidable cause of death worldwide, with increasing rates in adults and children. Trends in obesity prevalence proportions reveal the number of type II diabetes cases are expected to rise by 32% in Europe, 72% in the United States and over 100% in developing countries (Figure1.1) (1,2). Particularly, Among Asia-pacific nations, Thailand is top five with the highest number of obese people. Thailand will have approximately 21 million

overweighed people by 2015 according to the Ministry of Public Health (3). “Overweight” and “obese” are defined as abnormal or excessive fat storing that presents a risk to health (4). In human, adipose tissue is loose connective tissue composed of adipocytes. The fat may be deposited in other tissues such as muscle, heart, liver and pancreas, etc. The excess lipids accumulation in ectopic tissues leads to cell dysfunction or cell death (5). This phenomenon, known as lipotoxicity, can induce inflammation which plays a vital role in pathogenesis of diabetes and vascular disease in humans (6, 7). Adipocytes in white adipose tissue are the major contributor to obesity, insulin resistance and type II diabetes.

The major function of white adipocytes is to maintain lipids glucose and energy balance in the body by converting glucose into triglyceride (8). Moreover, adipocyte as an endocrine organ can secrete hormones and cytokines called adipocytokine or adipokine. Currently, many types of adipokine are found e.g., tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), leptin, adiponectin and resistin. These compounds play important roles in the normal development of cell types (cell differentiation), energy metabolism. Yet, they remarkably involve in the incidence of insulin resistance since people who have an extensive accumulation of fat in body are found to secrete several adipokines in large amounts (9).

Obesity is associated with changes in morphology of adipocyte. As obesity develops, adipocytes undergo hypertrophy (cell size increase) and hyperplasia (cell number increase) owing to increase storage of triglyceride (10). The number of fat cells in normal adults is strictly controlled by about 3 billion cells. The adipocyte cells turnover number in humans vary greatly from a low of 10% per year to a high of greater than 60% per year (11). In previous studies, the amount of fat normally depends upon only the size, but not the number of fat cells (11, 12). However, in obese people, both the fat cell size and numbers can be increased in order to accommodate the limitation of fat cell size to store excess fat from over-nutrition (12, 13). Upon losing weight, only the size of the cells is decreased, but the number of fat cells remains constant (12). Therefore, people who are

overweight always have trouble in losing weight whether by restricted diet or exercise (10, 11). Interestingly, control of proliferation and differentiation of fat cells will be a new target for the treatment of obesity.

1.2.2 Adipocyte life cycle

Life cycle of adipocyte (Figure 1.2) can be originally derived from mesenchymal stem cell (14, 15). Mesenchymal stem cells are the precursors of several different cell types, including chondroblasts, osteoblasts, myoblasts and preadipocytes. Primary preadipocytes are morphologically similar to fibroblasts, and unable to synthesize/accumulate fat within the cells. Upon clonal expansion and maturation, preadipocytes proliferate and change their morphology to become mature adipocytes. It became round to oval shaped cells with a distinct cell boundary and a nucleus set to one side due to the accumulation a lot of fat in the cells. Role of adipogenic factors in regulating the switch from clonal expansion to differentiation during adipogenesis divide in to two stages. In proliferation stage cell were stimulated by G1 Cyclin/Cdks complex and E2F protein. It drive cell from G0 to G1/S phase so cell start to proliferate. This stage known as proliferation or clonal expansion and then CCAAT enhancer-binding protein-δ (C/EBPδ), C/EBPβ, Rb protein were expressed to induce the alteration of cell shape and expression of the master regulator of differentiation. There are two adipogenic transcription factors, C/EBPα and peroxisome proliferator-activated receptor-γ (PPARγ). The C/EBPα expression is early up-regulated and then promote the PPARγ expression. These PPARγ induce the expression of cyclin-dependent kinase inhibitor (CKI). This inhibitor can trigger a post mitotic growth arrest stage of maturing preadipocyte. Following growth arrest, both C/EBPα and PPARγ transcription factors mediate the adipocyte differentiation through an up-regulation of the expression of various genes that contribute to the synthesis and accumulation of fat within the cells (16). Mature adipocytes can continue storing lipid when energy intake exceeds output. It can be hypertrophy depending on the

amount and accumulation of fat. Normal turnover of fat cells is controlled by apoptosis, so the net number of fat cells remains constant in the body.

To study adipocyte differentiation, many previous *in vitro* studies have extensively used two types of immortalized cell lines, 3T3-L1 and 3T3-F422A cell lines. Both preadipocyte state cells lines are stem cells destined to adipocyte differentiation (17). 3T3-L1 cells can be differentiated into adipocytes by addition of hormone mixtures composed of 3-isobutyl-1-methylxanthine (IBMX), dexamethasone and insulin. These agents are important to stimulate the expression of various genes, especially transcription factors that cause the differentiation of preadipocytes into mature fat cells. Ultimately, the accumulations of triglycerides are found in mature adipocyte (18).

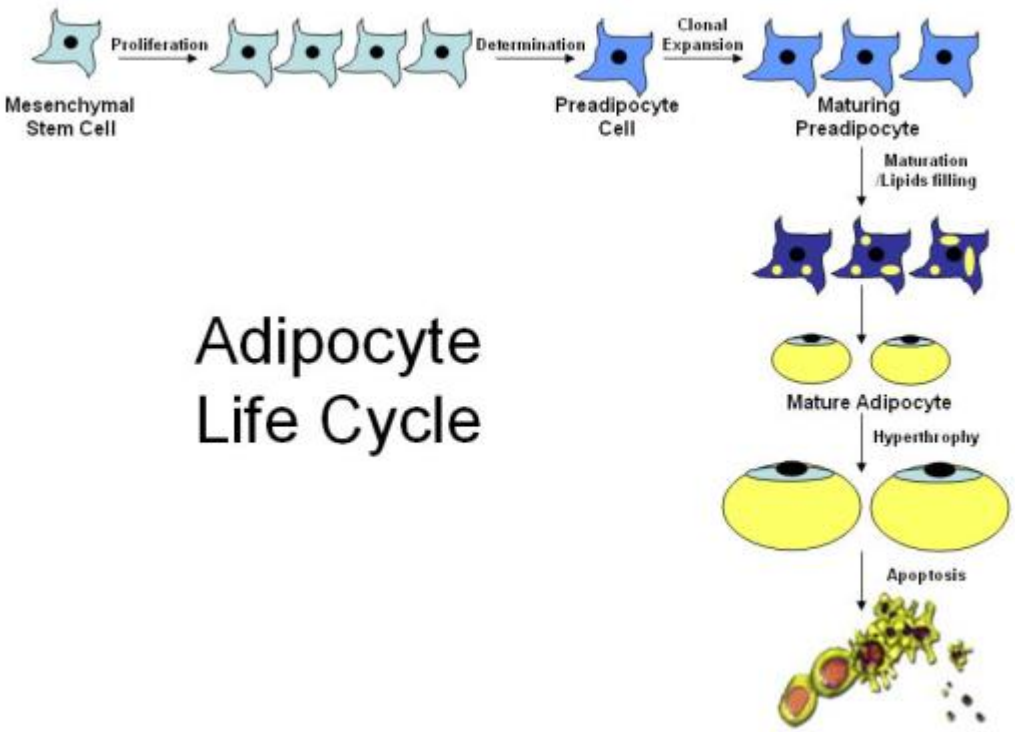


Figure 1.2 Life cycles of adipocytes (11)

1.2.2.1 Hormonal Inducers of Differentiation

Hormones and growth factors that affect adipocyte differentiation in many cell systems are insulin, insulin-like growth factor 1 (IGF-1), growth hormone, glucocorticoid and cAMP (table 1). In 3T3-L1 cells a mixture of insulin, dexamethasone, fetal bovine serum and 3-isobutyl-1-methylxanthine induce differentiation in post-confluent 3T3-L1 preadipocytes. Insulin and IGF-1 appear to play specific roles in adipocyte differentiation. Insulin and IGF-I mediates its adipogenic actions via IGF-I receptor. Several signal transduction pathway leads to the adipogenesis exerted by its binding. Glucocorticoids are the key hormone to stimulate 3T3-L1 cell differentiation. Dexamethasone can act as the glucocorticoid for binding the glucocorticoid receptor (GR). It induces C/EBPδ expression which resulted in its adipogenic activity (19). Glucocorticoid also has important role in the inhibition of phospholipase A2 leads to reduced prostaglandin induced the suppression of 3T3-L1 differentiation (20). Growth hormone can be induced the adipogenesis in preadipocyte (20). Fetal calf serum also provides the growth hormon needed for 3T3-L1 cells differentiation. IBMX is phosphodiesterase inhibitor. It can increase intracellular cAMP and dexamethasone is glucocorticoid agonist. It can bind to the glucocorticoid receptor.

Table 1.1 Hormones and differentiation factors influencing adipocyte differentiation(21).

Agent	Effect	Comments
Insulin	+	Accelerates lipid accumulation
IGF-1	+	Stimulates adipocyte differentiation
Glucocorticoids	+	Stimulate adipocyte differentiation
Growth hormone	+/-	Induces adipogenesis in preadipose cell lines, inhibits adipogenesis in primary cultures
Retinoic acid	+/-	Concentration dependent
Thyroid hormone	+/no effect	Inducing effect on adipogenesis restricted to a preadipose cell line
Prostaglandins	+/-	Varied effects depending on model system
EGF, TGF-α	-	Inhibit adipocyte differentiation
TGF-β	-	Potent inhibitor of adipogenesis
aFGF, bFGF	+/-	Conflicting results
IL-1, interferon-γ, TNF-α	-	Inhibit adipocyte differentiation
PDGF	+/-	Conflicting results
cAMP	+	Induces adipocyte differentiation
Vitamin D	+/-	Conflicting results
Oestrogen, progesterone	+/no effect	

Abbreviations: IGF-1, insulin-like growth factor 1; EGF, epidermal growth factor; TGF, transforming growth factor; FGF, fibroblast growth factor; IL-1, interleukin-1; TNF-α, tumor necrosis factor-α; PDGF, plateletderived growth factor; cAMP, cyclic adenosine monophosphate.

1.2.2.2 The adipogenic transcription factors

The adipogenic transcription factors, C/EBPαand PPARγ, are the master regulator of differentiation (22). Both transcription factors up regulate genes involved in adipogenesis and lipogenesis. Moreover, they stimulate several adipocyte-specific genes encoding secreted factors, insulin receptor, and proteins related to the synthesis and binding of fatty acids e.g. adipose fatty acid-binding protein (ap2), acyl CoA oxidase, uncoupled protein 2 (UCP-2), acetyl-CoA carboxylase 1 (ACC1), fatty acid synthase (FAS). In adipocytes, three members of the C/EBP family regulate early phases of adipogenesis, α, β and δ. C/EBPα acts as an activator for many adipocyte genes, such as GLUT4, leptin and aP2 (23). C/EBPβ and C/EBPδ are

expressed early after induction of adipogenesis (24). C/EBPβ is induced by the cAMP Response Element Binding Protein (CREB) in response to changes in cAMP levels (25). PPARγ is responsible for activating many of the genes involved in fatty acid uptake and storage. The important role of PPARγ in adipocyte differentiation has been demonstrated through multiple experiments including *in vitro* overexpression and knockdown, as well as in vivo gene targeting in mice (26). Knowledge drawn from these experiments suggests that PPARγ is necessary and sufficient for adipogenesis. It is noteworthy that down regulation of the C/EBPα and PPARγ gene expression could inhibit adipogenesis and lipogenesis processes of adipocyte differentiation (22).

1.2.3 Natural product inhibited adipogenesis

Suppression of adipocyte differentiation likely leads to inhibition of adipogenesis and/or induction of fat cell apoptosis. Several reports have shown the effect of natural products including rice bran extract on the life cycle of adipocyte (Table 2) (27-29).

Table 1.2 Examples of natural products with anti-adipogenesis effect

Source	Mechanism
Resveratrol (Grape peel) (27) Berberine (alkaloid in traditional Chinese medicine) (28)	Inhibit preadipocyte proliferation
Genistein (fava bean,soy) (27)	Inhibit preadipocyte proliferation, Reduce the accumulation of fat in maturing preadipocytes, induce lipolysis and apoptosis in mature adipocytes
Epigallocatechingallate, EGCG (green tea) (29)	Inhibit preadipocyte proliferation andadipocyte differentiation
Quercetin (flavanol found in many fruits and vegetables such as green tea, apples, onions, tomatoes) (27)	Inhibit preadipocyte proliferation and apoptosis of preadipocytes, induce lipolysis mature adipocytes
Fermented rice bran extract (Fermented rice bran) (30)	Ameliorate oxidative stress induced by high glucose and hydrogen peroxide in 3T3-L1 adipocytes.

1.2.4 Obesity-induced chronic inflammation

A combination of excessive food or energy imbalance can trigger obesity-induced inflammatory changes in adipose tissue (31, 32), and in long term affects the metabolic activation signal continuously (33). The signals of a metabolic pathway are found cross-talk with the inflammatory signaling within the cell. The levels of many inflammatory cytokines such as TNF-α, IL-1β, IL-6, are significantly increased in the adipose tissue of obese animal (34). Most importantly, the other organs including the liver, pancreas, brain and heart were also exposed to increased inflammation caused by obesity (35-37).

Activation of inflammatory signaling in adipocyte as a result of nutrients will cause a gradual accumulation of the inflammatory cytokines (Figure 1.3) (31, 38). Activation of inflammatory intracellular signaling pathways is mediated via the downstream inflammatory response such as the c-jun N-terminal kinase (JNK), inhibitory-κB kinase (IKK), or protein kinase R (PKR) pathways. High fat diets include free fatty acid results in inflammatory signaling pathway such as activation of JNK in skeletal muscle and liver tissues (39). A study in humans showed that levels of reactive oxygen species (ROS) were increased leading to induction of nuclear factor κ-B (NF-κB) in leukocyte within a few hours after ingestion of a high-fat, high-carbohydrate meal (40, 41). Therefore, the signal stimulates inflammation occurs in both early and late stage after receiving nutrients. Activation of signal occurs continuously for a long time could be called a chronic inflammation. It can activate the white blood cells in the immune system, such as macrophage, T cell and mast cell and also induces the macrophage infiltration into adipose tissue. This chronic process in adipose tissue results in a stronger pro-inflammatory response and lead to inhibition of metabolic cell function (42-46).

The results of several studies in human support the theory of obesity-induced inflammation. For example, in obese people, the secretion of inflammatory cytokine, the action of protein kinase and the amount of

macrophage cells are increased within adipose tissue compared to non-obese control group (47). Furthermore, one study found that increasing levels of inflammatory protein such as C-reactive protein (CRP) and leukocyte in humans are involved with an increased risk of developing type 2 diabetes (48). Moreover, large-scale genetic analyses have indicated significant systemic connections between obesity and inflammatory gene networks (49).

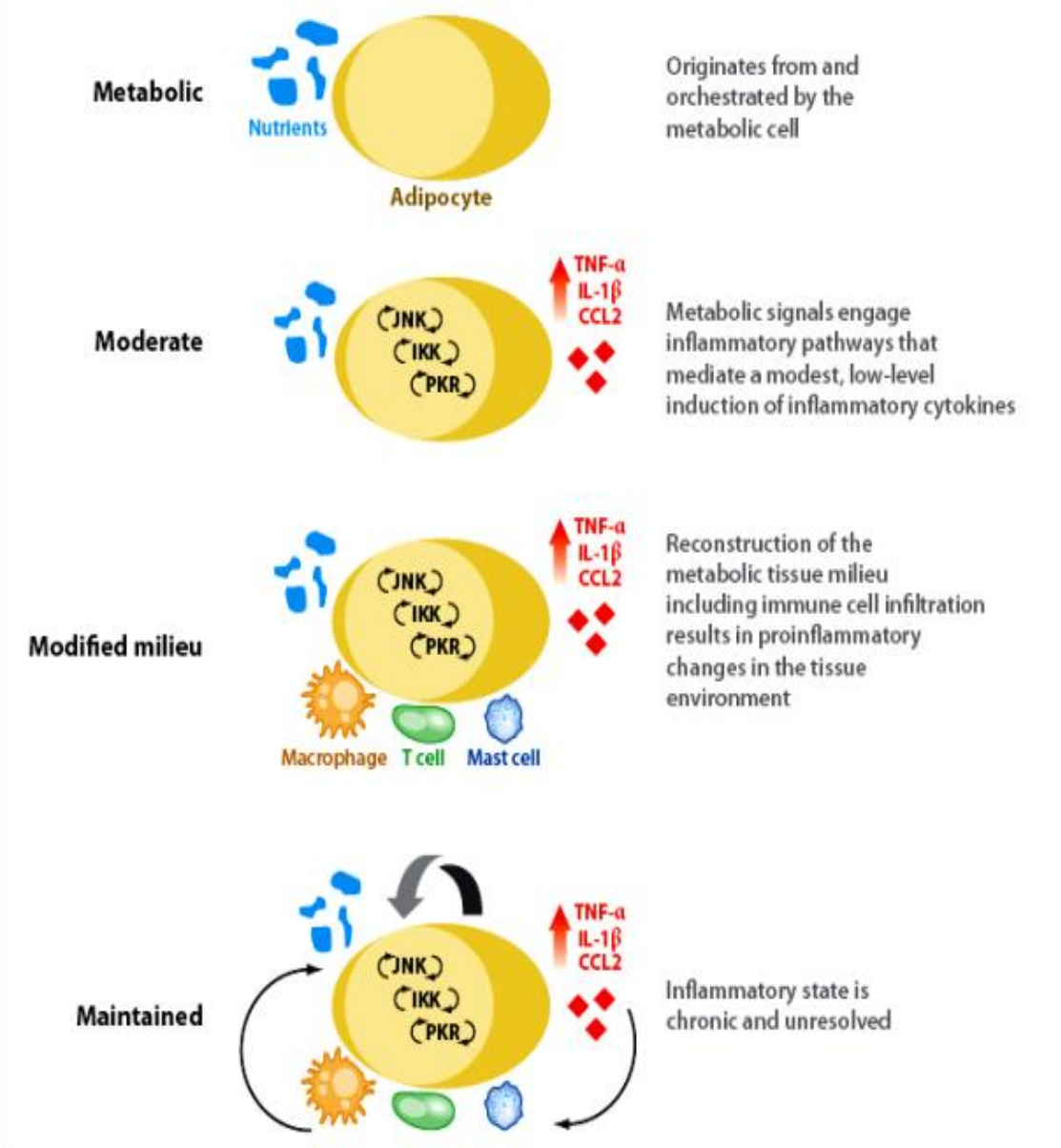


Figure 1.3 Obesity-induced chronic inflammations in adipocyte (38)

1.2.5 Inflammation induced insulin resistance

Several disease or conditions make body tissue resist to the insulin action such as infection, stress, severe illness, pregnancy or during using steroid. Some drugs can also contribute to the insulin resistance. Besides the most important cause of insulin resistance, inherited genetic factor, insulin resistance is often seen in people with metabolic syndrome; a group of conditions associated with excess weight and obesity (particularly visceral adiposity), hypertension and high levels of triglycerides and cholesterol in the blood. Visceral adiposity increase the secretion of inflammatory cytokines such as TNF-α, IL-1 and IL-6, both from visceral adipocyte cells and infiltrated immune cell (34). This inflammatory state of visceral adiposity is chronic and unresolved (38).

Cytokine that is generated from inflammationin adipocytes such as TNF-α can cause resistant to insulin (insulin resistance). TNF-α induced a mechanism involved in a tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) via JNK, IKK and PKR. This tyrosine phosphorylation can inhibit normal pathway of insulin signaling transduction for cellular glucose uptake (50, 51). These kinase are not only inhibit insulin signaling, but also control of many transcription factors, including the activator protein-1 (AP-1), NF-κB, interferon regulatory factor (IRF). These transcription factors will induce the other pro-inflammatory cytokine gene such as IL-1 and IL-6 expression in both adipocyte and macrophage. These cytokinescan be secreted to the circulation and will more promote the inflammatory response of peripheral tissue to increase further inhibition of whole body insulin signaling transduction (52, 53).

Recent study has indicated that inflammation in adipocyte not only limited function of insulin in glucose uptake. Secreted TNF-α in inflammation-adipocyte can cause lipolysisbyinhibition of the anti-lipolytic action of insulinand lead to free fatty acid secretion and the pathogenesis of adipocytes (54). Moreover, TNFα-mediated signaling pathways might down regulate PPARγ protein expression.

PPARγ is a master regulator for adipogenesis and lipogenesis. Therefore, its disruption decreases triglyceride storage and insulin sensitivity in adipocytes due to reduced levels of adiponectin, a cytokine that plays a key role in controlling the response of insulin in adipocytes. A low chronic inflammation of obese adipocyte results in stress condition lead to insulin resistance, lipolysis and adipocyte dysfunction. These in turn induce more chronic inflammation both in adipocyte and macrophage and lead to systematic inflammation and insulin resistance. Food can be useful as the most powerful medicine available to heal chronic disease. Consumption of foods with high anti-inflammatory properties such as polyunsaturated oils may be a wise option for prevention and treatment of metabolic syndrome. Omega-3 fatty acids such as eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) have been proven to reduce inflammation (55). In previous study, rats fed with polyunsaturated fatty acids-supplemented diets had decreased levels of the inflammatory cytokine expression and lower number of macrophage cells in adipose tissue, while the insulin sensitivity was improved in the liver and muscle (56). Moreover, studies in humans suggested that consumption of omega-3 could reduce the risk of heart disease and lower blood triglyceride levels in patients with diabetes (57). However, other studies found no relationship of omega-3 fatty acids in preventing or treating of obesity and diabetes.

1.2.6. Glutinous rice grains local varieties (*Oryza sativa* L.)

1.2.6.1 Local Purple rice variety

Purple glutinous rice is commonly known as "Kao Kum" or black sticky rice in the northern region of Thailand. Its name comes from the sticky shiny purple colored texture of the brown rice. In Thailand, purple rice genotypes, rice with purple pigment in the husk (hull) and pericarp, are local races. The type is a glutinous *indica*, grown widely in different geographical areas across country and varied in their phenotype pigmentation. Three varieties of purple rice used in this study are Doisaket (DSK), Nan (NAN) and Phayao (PYO). There are no differences in the

phenotype of this three varieties. The appearances of purple rice including leaf blade, leaf sheath, internode, ligule and auricle are similar in color, except node and internode of NAN are less color than DSK and PYO (table 1.3) (58). Karladee *et al.* (2007) has been reported the genetic relationship of 25 rice varieties including DSK, NAN, PYO and RD6 using a set of 5 simple-sequence-repeat (SSR) markers. The study showed that DSK and PYO are in the same genetic clustered. NAN is not in the same genetic cluster with DSK and PYO (fig 1.4) (59).

Table 1.3 phenotype of purple rice in this study (58)

varieties	leaf blade	leaf sheath	node	internode	ligule	auricle
DSK	purple	purple	purple	purple	Light purple	Light purple
PYO	purple	purple	purple	purple	Light purple	Light purple
NAN	purple	purple	colorless	Light purple	Light purple	Light purple

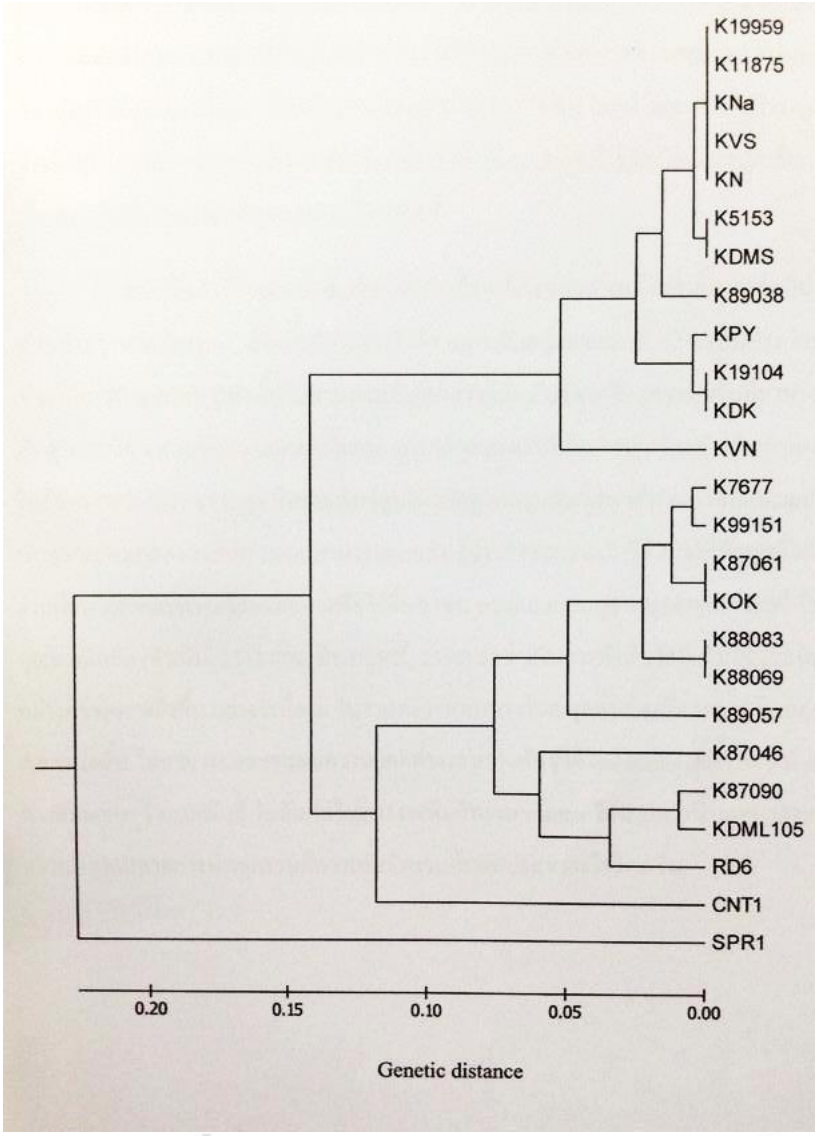


Figure 1.4 Genetic distance clustering by using 5 sets of simple sequence repeat analysis showing genetic relationship among 25 varieties including glutinous purple rice and white rice used in this study, abbreviations: KN, Nan; KPY, Phayao; KDK, Doisaket; RD6 (59)

1.2.6.2 Commercial white rice variety

The commercial white rice variety used in this study is Rice department variety 6 (RD6). The polished RD6 rice is white in color. But this study use it in a form of unpolished grain, the color appears in brown. RD6 has been planted as the main glutinous rice cultivar in northern part of Thailand. It is a glutinous mutant from a non-glutinous parent cultivar KDML105. It was developed by gamma rays seed irradiation. Rice Research Institute, Department of Agriculture, Ministry of Agriculture and Cooperatives officially approved and released to the farmer in 1977 (60.)

1.2.7 Rice and active ingredients

Presently, there is no effective treatment against obesity and insulin resistance, therefore bioactive compounds and natural products that exhibited anti-obesity effects are of intense medical interest. Rice (*Oryza sativa* L. indica) can be found in more than forty thousand varieties throughout the world. Whole grain rice is high in nutritional value because of their biologically active constituents including anti-oxidants, fiber, minerals and phytoestrogens (61, 62) whereas white rice is processed to remove its bran, leaving it with less nutritional value. Major constituents of unpolished brown rice are shown in figure 1.5 (63). The major component of the endosperm is 77% carbohydrate (starch), 6% protein and 0.9% fat and minerals. Dietary fiber is highest in the bran layer. Minerals (ash) are also concentrated in the outer layers of brown rice or in the bran fraction. Therefore milling affects the nutritional quality of the rice. Milling strips off the bran layer, leaving a core contains mostly carbohydrates. Moreover compositions of microcomponents of rice bran are shown in figure 1.6. The main compositions of microcomponents in rice bran are mineral, amino acid, inositol and γ-oryzanols, respectively. There are several microcomponents exhibited as an antioxidant such as inositol, tocopherols (vitamin E), tocotrienols, γ-oryzanols, polyphenols (ferulic acid, α-lipoic acid, *p*-sinapic acid) These antioxidants are present in different concentrations in all three rice bran fractions, stabilized rice bran, rice bran water solubles, and rice bran fiber concentrates. These antioxidants might be able to

maintain glucose levels by exerting their effects on glucose absorption, utilization, and excretion (63).

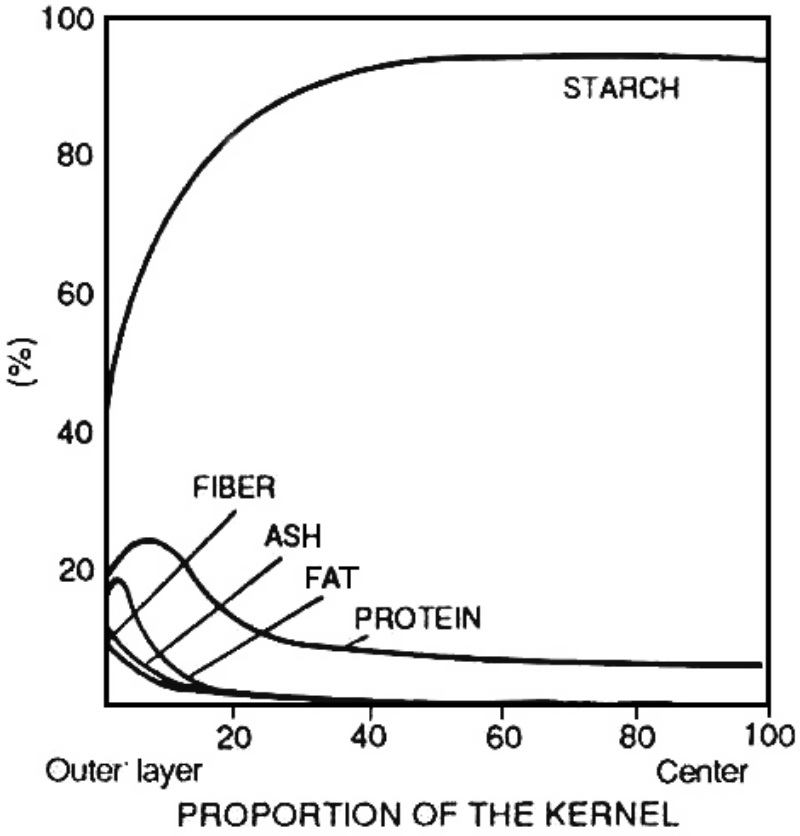


Figure 1.5 Distribution patterns of major constituents of brown rice (63)

Carotenoids
0.9–1.6 ppm

α-Carotene
β-Carotene
Lycopene
Lutein
Zeaxanthine

Tocopherols/Tocotrienols
(Vitamin E) 210–440 ppm

α-Tocopherol
β-Tocopherol
γ-Tocopherol
δ-Tocopherol
α-Tocotrienol
β-Tocotrienol
γ-Tocotrienol
δ-Tocotrienol
Desmethyl Tocotrienol
Didesmethyl Tocotrienol

Polyphenols
305–390 ppm

Ferulic acid
α-Lipoic acid
Methyl ferulate
p-Coumaric acid
p-Sinapic acid
Quercetine
Isovitexin
Proanthocyanidins
Caffiec acid
cinnamic acid

Other antioxidants
(ppm)

Inositol (1100–1400)
Myoinositol (1000–1200)
Choline (930–1150)
Phytates (1500–1710)
Biotin (0.1–0.22)

Vitamin-B Complex
(ppm)

Niacin (370–660)
Pantothenic acid (36–50)
Pyridoxin (29–42)
Thiamin (22–31)
Riboflavin (2.2–3.5)

Phytosterols
2230–4400 ppm

β-Sitosterol
Campesterol
Stigmasterol
Δ5 Avinsterol
Δ7 Stigmasterol

Gramisterol
Citrostdienol
Obtusifoliol
Branosterol
28-Homotyphasterol
28-Homosteasteronic acid
6-Deoxycastasterone
β-Amyrin

Enzymes

Glutathione peroxides
Methionine reductase
Superoxidase dismutase
Polyphenol oxidase
Coenzyme Q10

γ-Oryzanols
2200–3000 ppm

Cycloatenyl ferulate
Campesteryl ferulate
Stigmasteryl ferulate
β-Sitosteryl ferulate
24-Methylene cyclartanyl ferulate

Phospholipids

Phosphatidylcholine
Phosphatidylethanolamine
Lysophosphatidylcholine
Lysophosphatidylethanolamine

Polysaccharides

Cycloartenol ferulic acid glycoside
Diferulic acid complex
Diferulic acid + 3 Glucose + 2-Calcium complex

Amino Acids

Arginine (10800 ppm)
Histidine (3800 ppm)
Methionine (2500 ppm)
Tryptophan (2100 ppm)
Cystein (336–448 ppm)
Cystine (336–448)

Minerals

Magnesium (6250–8440 ppm)
Calcium (303–500 ppm)
Phosphorous (14700–17000 ppm)

Figure 1.6 Composition of microcomponents including antioxidants of stabilized rice bran, rice bran water solubles, and rice bran fiber concentrates (64)

In previous studies, unpolished white rice grain or brown rice has been shown to ameliorate glucose intolerance and protect the onset of diabetes in mice fed a high-fat diet (65). γ-oryzanol is one of the major bioactive components in brown rice (66). It shows cholesterol-lowering, anti-inflammatory, anticancer, anti-diabetic, and antioxidant activities (67). In addition, germinated brown rice (GBR) is potential agent against obesity (68). It control suppressed body weight gain and lipid accumulation in the liver and epididymal adipocyte by controlling adipogenesis through a reduction in transcriptional factor (68). Moreover, purple rice (*Oryza sativa* L.) has anthocyanin in the purple pigment found in the husk (hull) and pericarp. Anthocyanin has various excellent biological activities including antioxidant, anti-inflammation, anti-cancer, and specifically hypoglycemic effect (69).

Many kinds of food additives and plant possess anti-inflammatory activity. Colorful fruits and vegetables are excellent sources of phenolic compounds. The dietary phenolics can be divided into phenolic acids, flavonoids, and polyphenols (Figure 1.7). Anthocyanins, a flavonoid component of the pigment are found in many plant families, such as berries, apples, grapes and colored rice. There are over 300 structurally different anthocyanins that have been identified in nature. The anthocyanins are composed of anthocyanidins with sugar group(s), generally3-glucosides. Anthocyanins in nature are the glycosides of cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin (Figure 1.8). These compounds were converted to proanthocyanidins, also known as condensed tannins, which are dimers, oligomers, and polymers of catechins links together between C4 and C8 (or C6).

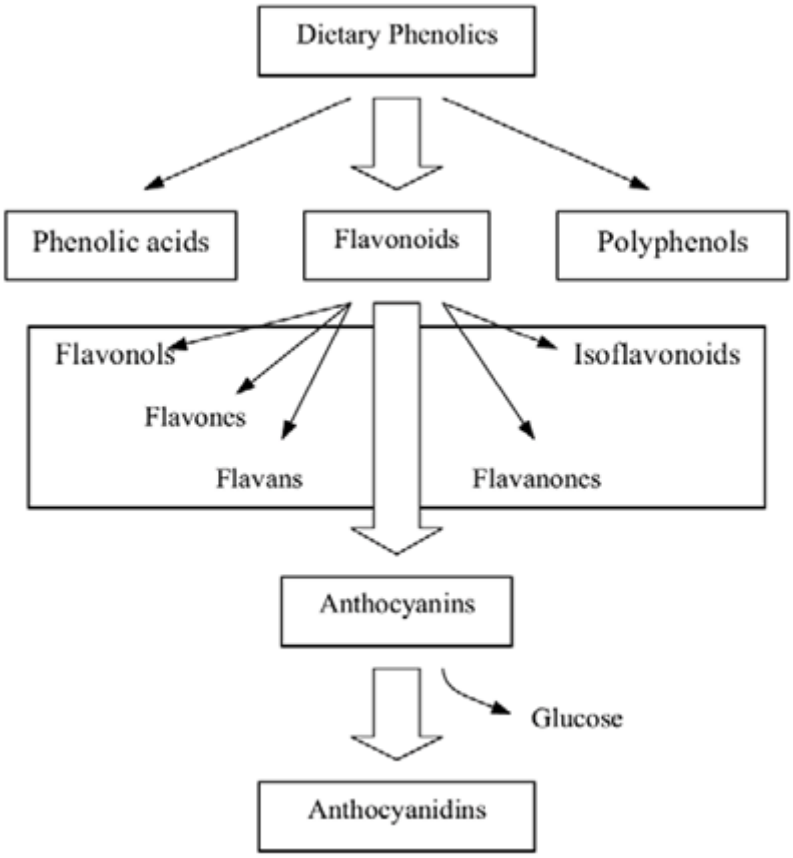
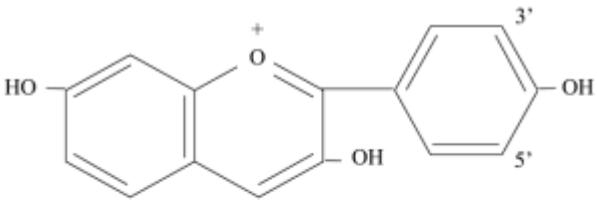


Figure 1.7 Group of phenolic compounds in foods



Anthocyanidins:

Compound	Carbon ring B substitution	
	3'	5'
Pelargonidin	-H	-H
Cyanidin	-OH	-H
Delphinidin	-OH	-OH
Peonidin	-OCH3	-H
Petunidin	-OCH3	-OH
Malvinidin	-OCH3	-OCH3

Figure 1.8 Structures of anthocyanin (70)

Anthocyanins can act as powerful antioxidants, so they effectively inhibit the formation of free radicals that are cause of pathology in various diseases, including cardiovascular disease, cancer and diabetes (71). Anthocyanins can also help control of obesity and insulin response through PPARγ activation and cytokine secretion, including adiponectin and leptin from the adipocyte (72, 73). In addition, these compounds play a role in reduction of inflammation by inhibiting production of nitric oxide (NO) and cyclooxygenase (COX) enzymes. One study in 2010 found that anthocyanins derived from black rice are cyanidin-3-O-beta-D-glycoside and cynanidin. These compounds can inhibit adipocyte inflammation both *in vitro* and *in vivo* by reducing both the expression of cytokines, TNF-α and IL-1β , and the synthesis of NO and prostaglandin E2 (PGE2), a major inflammatory mediator. Compounds derived from black rice can also reduce the expression of COX and nitric oxide synthase (iNOS) via NF-kB pathway (74). Besides anthocyanin, black rice contains other substances such as metabolite of linoleic acid that can inhibit inflammation (75, 76). Moreover, another has found that grape seed proanthocyanin can stimulate adiponectin and reduce IL-6, resulting in inhibition of adipocyte inflammation (77). Therefore, extracts of rice is likely to be effective in inhibiting the inflammation of adipocyte and improving glucose homeostasis.

1.3 Obectives of the study

1.3.1 To determine the cytotoxicity of the dichloromethane extract and the methanol extract of purple rice, DSK, NAN, PYO and unpolished white rice RD6 on 3T3-L1 adipocyte cell.

1.3.2 To determine the effect of the dichloromethane extract and the methanol extract of purple rice, DSK, NAN, PYO and unpolished white rice RD6 on the adipogenesis of 3T3-L1 preadipocyte cell.

1.3.3 To determine anti- insulin resistant activity of the dichloromethane extract and the methanol extract of purple rice, DSK, NAN, PYO and unpolished white rice RD6 in 3T3-L1 adipocyte cells.