ANTIHYPERGLYCEMIC AND TOXICOLOGICAL STUDIES OF ETHANOLIC–AQUEOUS EXTRACTS OF *GYNURA PROCUMBENS*

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UNIVERSITI SAINS MALAYSIA

June 2013

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By

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Thesis submitted in fulfillment of the requirement for the degree of the Doctor of Philosophy

2013

ACKNOWLEDGEMENTS

I would like to express my utmost and deepest gratitude and appreciation to my wonderful supervisor Professor Mohd Zaini Asmawi for his towering presence.continuous effort, guidance and patience. His valuable experience was of tremendous help and was always a source of ideas. My constant discussions were always fruitful and has a played an important role in making this thesis reality.

My heartfelt gratitude and appreciation go to my co-supervisors, Associate Professor Norhayati Ismail and Professor Amirin Sadikun who have helped a lot with valuable inputs with regard to extraction and supply me the plant, without their contributions this thesis would have been incomplete.

I am most grateful to my wife and my children Fawaz and Rawan for their love, support, kindness, understanding and above all their patience which always provided me the necessary drive and inspiration to work hard.

I am indebted to my parents who provided me good education, very supportive and always being there for me. I am deeply grateful to my brothers and sisters for their kind support and encouragement.

My sincere thanks and appreciation to my laboratory technician Mr. Roseli Hassan, he has extended all possible help and has always made laboratory work easier. Thanks are also due to Mr Samsudin, Mr. Razak, Mr. Hamid. Mr. Rahim, Mr. Hassan, Mr. Yusof and Mr. Adnan in the animal house.

My sincere thanks also go to my friends and laboratory mates to support me specially Mahfouz, Beh, Lay Jing, Yam. Item, Ali and Elsnoussi with whom I have spent considerable time in the laboratory and were like family.

I wish to extend my thanks to University of Hadhramout for Sciences and Technology, Yemen for allowing and providing me with fellowship to get PhD.

I would like to express my appreciation to Prof Syed Azhar Sulaiman Dean of School of Pharmaceutical Sciences., USM for allowing me to use all facilities and equipments during these years.

Lastly, I offer my thanks and blessings to Malaysia, the great land with peace and kind people who give me a lot of things under the shadow of the safe, peace and love.

KHALED

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LIST OF ABBREVIATIONS AND SYMBOLS

%	percent
°C	degree centigrade
µg/mL	microgram per milliliter
µl	microlitre
µg	microgram
Abs	absorbance
ACR	acarbose
A.D.	anno domini
ADI	acceptable daily intake
ADP	adenosine diphosphate
ALK	alkaline phosphatase
AST	aspartate aminotransferase
ALT	alanine aminotransferase
ANOVA	analysis of variance
ATP	adenosine triphosphate
AUC	area under the curve
B.C	Before Christ
B.W	body weight
BSA	bovine serum albumin
cAMP	cyclic adenosine monophosphate
CCl_4	carbon tetrachloride
CMC	carboxymethyl cellulose
CV	coefficient of variation

DNA	deoxyribonuclueic acid
DMSO	dimethylsulphoxide
DNS	dinitrosalicylic acid
DNA	deoxyribonucleic acid
D M	diabetes mellitus
DW	distilled water
DPP-iv	dipeptidyl peptidase IV
EC_{50}	effective concentration 50 %
Ear	estimated average requirement
Eth-OH	ethanol
FFA	free fatty acids
fl	fimtoliter
GAE	gallic acid equivalent
GC-MS	gas chromatography-mass spectroscopy
GLP 1	glucagon-like peptide-1
GLUT 4	glucose transporter 4
GK	glucokinase
G6P'Tase	glucose 6 phosphatase
GOT	glutamate oxaloacetate transaminase
GLP-1	glucagon-like peptide 1
G. procumbens	*Gynura procumbens*
GPT	glutamate pyruvate transaminase
HbA	Hemoglobin A

HPLC	high performance liquid chromatography
HPTLC	high performance thin layer chromatography
Hr	hour
HDL	high density lipoprotein
HOMA	homeostasis model assessment
IgG	Immunoglobin G
IC_{50}	Inhibition concentration 50%
ip	intra peritoneal
IPGTT	intra-peritoneal glucose tolerance test
IR	Insulin receptor
IRSs	Insulin receptor substrates
Kg	kilogram
LD_{50}	lethal dose 50%
LOD	limit of detection
LOQ	limit of quantitation
µg	microgram
NADH	nicotinamide adenine dinucleotide
NIDDM	Non–insulin-dependent diabetes mellitus
NeAG	neoandrographolide
NO	nitric oxide
NOAEL	no observed adverse effect level
NC	normal control
mg	milligram

min	minute
mmol/l	milimole per liter
OECD	Organisation for Economic Co-operation and Development
OGTT	Oral glucose tolerance test
PBG	Peak blood glucose
pg	Picogram
PI	Phosphatidylinositol
PKB	Protein kinase B
RSD	Relative standard deviation
ROS	Reactive oxygen species
SEM	Standard error of mean
SD	Sprague Dawley
SPSS	Statistical procedures for social sciences
STZ	Streptozotocin
SGTT	Subcutaneous glucose tolerance test
ROW	Relative organ weight
RSD	Relative standard deviation
Umol/l	Unit mol per liter
UV-VIS	Ultraviolet-visible
v/v	Volume to volume
WHO	World Health Organisation
w/v	Water to volume

KAJIAN ANTIHIPERGLISEMIK DAN KETOKSIKAN EKSTRAK AKUES – ETANOL *GYNURA PROCUMBENS*

ABSTRAK

Daun *Gynura procumbens* (sambung nyawa) telah digunakan dalam perubatan tradisional Melayu untuk merawat diabetes. Adalah difahami bahawa daun mengandungi banyak sebatian dan hanya satu atau sebahagian daripadanya mempunyai aktiviti antidiabetik. Oleh itu, tujuan kajian ini adalah untuk mengekstrak daun menggunakan pelarut berbeza kepolaran, campuran nisbah yang berbeza antara etanol-air bagi memilih ekstrak yang mengandungi sebatian dengan aktiviti antidiabetik tertinggi dan menjalankan ujian ketoksikan akut dan subkronik terhadap ekstrak yang mempunyai aktiviti tertinggi. Aktiviti antidiabetik ekstrak-ekstrak dibanding berdasarkan kebolehan ekstrak-ekstrak menurunkan paras glukosa darah tikus normal dan tikus diabetik aruhan streptozotosin. Serbuk daun tumbuhan yang dikeringkan diekstrak menggunakan etanol 0, 25, 50, 75 dan 95% dan dipekatkan menggunakan penyejat berputar, masing masing. Ekstrak ekstrak etanol 0, 25, 50, 75 dan 95% menurunkan secara signifikan ($p<0.005$) paras glukosa darah tikus diabetik aruhan streptozotosin pada jam ke – 3 dan 5 selepas pemberian secara oral 1 g/kg ekstrak. Ekstrak 25% etanol menunjukkan aktiviti antidiabetik tertinggi dan sama seperti metformin menurunkan secara signifikan ($p<0.005$) paras glukosa darah pada jam ke -2, 3, 5 dan 7 selepas pemberian. Bagi analisis fitokimia, ekstrak etanol 75% mengandungi kandungan flavanoid tertinggi, sementara ekstrak air menunjukkan nilai terendah. Tambahan pula, fraksi n-butanol daripada ekstrak etanol

25% menunjukkan nilai tertinggi kandungan flavonoid dan fenolik. Kandungan fenolik tertinggi didapati dalam ekstrak etanol 50%. Kandungan asid klorogenik menunjukkan paras tertinggi dalam ekstrak etanol 70%. Ekstrak etanol 25% disisih menggunakan pelarut berbeza kepolaran seperti heksana, etil asetat, klorofom, n-butanol dan air. Aktiviti antihiperglisemik bagi setiap fraksi kemudian dinilai melalui pemberian akut dan berulang ekstrak etanol 25% *G. procumbens* dan fraksi – fraksi pada dos 500, 1000, 2000 mg/kg selama 14 hari dan kesan ekstrak dan fraksi – fraksi terhadap enzim α- glukosidase and α- amilase (*in vivo* dan *in vitro*) juga dikaji. Keputusan menunjukkan fraksi n-butanol mempamerkan kesan antihyperglisemic yang lebih kuat berbanding fraksi etil asetat dan akues. Hasil kajian menunjukkan bahawa pecahan butanol mempunyai kesan tertinggi pembetulan terhadap hiperglisemia setelah makan siang selepas pemberian kanji dan sukrosa dalam tikus normal dan diabetik oleh perencatan aktiviti α-glucosidase dan α-amilase. Penemuan ini menunjukkan bahawa fraksi n-butanol mampu mempamerkan kesan penurunan glukosa yang signifikan pada dos 1000 mg/kg sama seperti metformin 500mg/kg disebabkan kehadiran komponen aktif polifenolik seperti asid klorogenik, rutin dan kemferol -3-O-rutinosid. Tambaban pula, hal ini mencadangkan bahawa fraksi n – butanol mempunyai kesan antihiperglisemik yang lebih baik berbanding fraksi etanol 25% *G. procumbens*. Akhir sekali, kajian ketoksikan akut dan subkronik menunjukkan bahawa ekstrak etanol 25% *G. procumbens* boleh dianggap tidak mempunyai sebarang risiko toksik.

Kata kunci: *Gynura procumbens*, aktiviti antidiabetik, antihiperglisemik, perencat α-glukosidase, ketoksikan akut dan subkronik.

ANTIHYPERGLYCEMIC AND TOXICOLOGICAL STUDIES OF ETHANOLIC– AQUEOUS EXTRACTS OF *GYNURA PROCUMBENS*

ABTRACT

Leaves of *Gynura procumbens* (sambung nyawa) have been used in Malay traditional medicine among others to treat diabetes. It is understood that the leaves contain many compounds and only one or a few of these compounds possess the antidiabetic activity. Therefore, the aim of this study was to extract the leaves using solvents of different polarity, mixtures of the different ratio of ethanol-water to select the extract that contains compound(s) with the strongest antidiabetic activity and to carry out acute and subchronic toxicity tests of the extract with better activity. The antidiabetic activity of the extracts was compared based on their ability to lower down the blood-glucose level of normal rats and streptozotocin-induced diabetic rats. Dried powdered leaves of the plant were extracted with 0, 25, 50, 75 and 95% ethanol and concentrated using rotary evaporator respectively. The 0, 25, 50, 75 and 95% ethanol extracts respectively significantly reduced ($p<0.005$) blood-glucose level of streptozotocin induced diabetic rats at 3rd and 5th hours after oral administration of 1000 mg/kg of the extracts. The 25% ethanol extract showed the strongest antidiabetic activity and similar to metformin significantly reduced ($p<0.005$) blood-glucose level at the 2nd, 3rd, 5th and 7th hours after administration.

In the phytochemical analysis the 75% ethanolic extract contained the highest flavonoids content, while the water extract showed the lowest value. In addition, the n-butanol fraction from 25% ethanolic extract represented the highest value of flavonoids and phenolics contents. The highest phenolics content was found in 50 %

ethanolic extract, although chlorogenic acid content showed the highest level in 75% ethanolic extract. The 25% ethanolic extract was fractionated with solvents of differing degree of polarity such as hexane, ethyl acetate, chloroform, n-butanol and water. The antihyperglycemic activity each fraction was then assessed by acute, repeating administration of 25% ethanolic extract *of G. procumbens* and fractions at doses of 500, 1000 and 2000 mg/kg respectively for 14 days and also, by investigating the effect of the extract and its fraction on α-glucosidase and α-amylase enzymes activities *in vivo* and *in vitro*. It seems that butanol fraction possess the highest corrective effect on postprandial hyperglycemia after administration of starch and sucrose in the normal and diabetic rats by inhibition of the α-glucosidase and α-amylase acivities. Furthermore, the results indicate that n-butanol fraction exhibited a stronger antihyperglycemic effect than the ethyl acetate and aqueous fractions.. These findings showed that the n-butanol fraction could exert a significant glucose lowering effect at dose 1000 mg/kg similar to that metformin 500 mg/kg due to the presence of polyphenolics active constituents such as chlorogenic acid, rutin and kaempferol-3-O-rutinoside. In addition, it suggested that the n-butanol fraction had a better antihyperglycemic and acumulative effects than the other fractions of 25% ethanolic *G. procumbens.* Finally, the acute and subchronic toxicity study showed that the 25 % ethanolic extract of *G. procumbens* can be considered devoid of any toxic risk.

Keywords: *Gynura procumbents*, antidiabetic activity, antihyperglycemic, α-glucosidase inhibitor, acute and subchronic toxicity

Chapter 1

Introduction

1.0. Diabetes Mellitus

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (DeFronzo, 2004). Without enough insulin, body tissues, in particular, the liver, muscle and adipose tissues fail to take and utilize glucose from the blood circulation. This results in elevated blood glucose levels, a condition known as hyperglycemia. If blood glucose levels remain high over a long period of time, this can result in long-term damage of organs such as the kidneys, eyes, nerves, heart and blood vessels. Complications in some of these organs can lead to death (Brownlee, 2001; Weiss and Sumpio, 2006).

Currently, type II diabetes mellitus, the most common type of diabetes mellitus, is managed by a combination of diet, exercise, oral hypoglycemic drugs and sometimes insulin injections (Day and Bailey, 2006). However, synthetic oral hypoglycemic drugs, which are currently the main form of treatment for type II diabetes mellitus have been shown to have undesirable side effects and high secondary failure rates (Alberti and Zimmet., 1998). In addition, these drugs cannot be afforded by the majority of people living in rural communities of developing countries such as South Africa because of their high cost (Day and Bailey, 2006). These limitations, of currently available antidiabetic pharmacological agents have prompted researchers all over the world to investigate alternative antidiabetic remedies. In particular, consideration is given to plants and herbs used by traditional healers and herbalists as antidiabetic remedies with the hope of discovering new natural products that can be used or developed into safe, inexpensive and effective antidiabetic remedies. In this context, a number of medicinal plants and herbs

have been studied and validated for their hypoglycaemic potential using experimental animal models of diabetes (Kesari et al., 2005; Ruzaidi et al., 2005) as well clinical studies involving diabetic patients. (Jaouhari et al.,1999; Herrera-Arellano et al., 2004; Jayawardena et al., 2005). In addition, bioactive compounds of most of these plants have been isolated and identified (Grover et al., 2002; Jayawardena et al., 2005). However, mechanisms of action whereby most of these plants and their products exert their blood glucose lowering effects on tissue or organs remain unknown.

Gynura procumbens (Lour) Merr, a composite known locally in Malaysia as Sambung Nyawa, is an annual evergreen shrub that grows extensively in South East Asia, particularly in Indonesia, Malaysia, and Thailand, where it is traditionally used for treatment of eruptive fevers, rash, kidney disease, migraines, constipation, hypertension, diabetes mellitus, and cancer . Some of these traditional claims have been validated in scientific and pharmacological studies, including, anti-herpes simplex virus, anti-inflammatory, and antihypertensive activities.

G. procumbens has of recent received particular attention in the pharmacology of antidiabetic medicinal plants, probably because of its avowed empirical evidence and efficacy in the traditional management of diabetes. However, the scientific reports on the antidiabetic activity of this plant have been conflicting and inconsistent. For instance, Zhang and Tan (2000) had reported that 95% ethanol extract improved glucose tolerance in STZ induced diabetic rats, but not in normal rats. Its aqueous extract was also reported by these authors to exert significant antihyperglycemic action in STZ-induced diabetic rats. Later on, Akowuah et al (2009) on the contrary indicated its glucose lowering effect in normal rats. In a most recent study, the extract of G. *procumbens* was reported to produce significant elevation in the fasting blood glucose levels of normal rats, but a decrease in diabetic rats. There is a basic need to stream line these reports, given the widespread traditional applications of use of G. *procumbens.* Moreover,

these study designs are not targeted at natural product discovery or production of standardized herbal forms. Adequate research on medicinal plants beyond screening for biological activity should be conducted with the aim to systematically standardize and develop them into natural products or dosage forms which should effectively complement or supplement existing conventional measures. Consequently, the present investigation using ethnomedical drug discovery program, evaluated the antidiabetic activity of *G. procumbens* used in the traditional health system of the South East Asia, as an effective remedy and management for diabetes mellitus and other ailments. A systematic screening such as this is a fundamental requirement for natural product exploration and development of therapeutic agents from medicinal plants.

1.1. The objectives of this study

1-To evaluate the antihyperglycaemic activities of ethanolic-aqueous extract of *G. procumbens* (95%, 75%, 50%, 25% ethanolic and water extracts) on normal and STZ induced diabetic rats.

2- To standardize the phytochemical constituents contained in the most potent antihyperglycemic extract.

3- To fractionate the most active antihyperglycemic ethanolic-aqueous extract and investigate the antidiabetic activity of the fraction in the normal and STZ induced diabetic rats.

4- To investigate the antidiabetic mode of action of the most bioactive extract and its fractions (ethyl acetate, n-butanol and aqueous) on inhibition of postprandial hyperglycaemia *in vitro* and *in vivo* animal models by studying the effect of the extract and fractions on α-glucosidase and α-amylase enzymes activity.

5- To evaluate the acute and subchronic toxicity of the most bioactive extract.

Chapter 2

Literature Review

2.0. Epidemiology and Incidence of diabetes mellitus

The growing seriousness of diabetes in the world can be based on the increase in patients, especially in developing countries. According to WHO reports, there are more than 180 million people worldwide suffering from diabetes, and these numbers are likely to double by 2030. In 2005, an estimated 1.1 million people died from diabetes, with approximately 80% of these deaths occurring in poor and developing countries. Almost half of these deaths was in people under the age 70 years with 55% being women. The WHO estimates that diabetes related deaths will increase by more than 50% in the next 10 years, if the disease is not given urgent attention. Most notably, diabetes deaths are projected to increase by over 80% in the upper-middle income between 2006 and 2015. Diabetes and its complications therefore pose significant economic and public health consequences for individuals, families, health system and countries (WHO, 2006).

Diabetes mellitus is a serious diseases affecting both poor and rich countries and also affecting both male and female as well as across racial lines. Obesity and lifestyle related to socio–economic status and nutrition of the people are the important risk factors that can increase the incidence of diabetes mellitus. In the world's highly industrialized countries, the prevalence is very high owing to the lush lifestyle the people are leading (Amos et al., 1997).

Diabetes mellitus is a growing health concern in Malaysia. The number of diabetic patients in Malaysia is increasing while complication rates and associated diseases among

diabetes are significantly high. Nowadays, low priority for a good eating habit and low awareness in health care represent the most problems in the people. Prevalence of diabetes mellitus in this country has also steadily increased over the years with an estimate of 0.65% in 1960, to 2% in 1982. Diabetes screening in the year 2002 showed that 80,204 cases were screened with 2,606 cases (65%) were normal, 14,271 (18%) cases were abnormal, and 13,327 (17%) cases were borderline case. Data in 2002 showed that 6,288 diabetes cases had their eye screened with undus cameras and the result showed that 4,150 cases or 66% was normal whereas 2,138 cases or 34% had some form of retinopathy (Ooyub et al., 2004). Currently there are around 1.2 million diabetes in Malaysia, with 98% of them diagnosed with diabetes mellitus type II. This means that there is approximately 8 diabetes in every 100 adults. The WHO has estimated that in 2030, Malaysia would have a total number of 2.48 million people inflicted with diabetes compared to 0.94 million in 2000 with an increase 164 % (PDM, 2007). This increase can be attributed to many factors, including a stressful lifestyle as well as improper dietary habits. This is of economic concern as the disease requires life-long treatment and is also associated with high morbidity from the resulting complications.

2.1. Classification of Diabetes Mellitus

Diabetes mellitus is classified into three major disease syndromes.

- Type I diabetes (previously known as insulin-dependent or childhood-onset) is characterized by a lack of insulin production. Type I diabetes is thought to result from an infectious or toxic environmental contingency in people whose immune systems are genetically predisposed to develop a vigorous autoimmune response against pancreatic β-cells antigen. There are many extrinsic factors that might affect β cells functioning, these factors include damage of these cells by certain

viruses such as the mumps virus and coxsakie virus B4, by chemical agents or by destructive cytotoxins and antibodies release from sensitized immunocytes. The degree of the damage of β-cells in diabetes mellitus I can be lessened at the initial manifestation by treatment with immunosuppressive drugs such as cyclosporine or azathioprine are (Nolte and Karam, 2001). The most frequent symptoms of diabetes mellitus include excessive urine production (polyuria) thirst (polydipsia), constant hunger despite the patient having a voracious appetite (polyphagia), weight loss, coma, voracious change and fatigue (WHO, 2006).

- Type II diabetes mellitus (called non- insulin dependent or maturity /adult-onset diabetes onset diabetes) results from the body‘s inability to use insulin, which results in its accumulation in the fat cells (WHO, 2006). It is the most common from of diabetes and is characterized by disorders of insulin action and insulin secretion, and either one can be the predominant feature (Alberti and Zimmet., 1998). Diabetes mellitus type II comprises 90 % of the cases of diabetes in people around the world and largely the result of excessive body weight and physical inactivity. Symptoms may be similar to those of diabetes mellitus type I but are often less marked. Diabetes mellitus type II is characterized by fasting and postprandial hyperglycemia. If left untreated hyperglycemia may cause long term microvascular and macrovascular complications, such as nephropathy, retinopathy and atherosclerosis. The pathogenesis in diabetes type II is that the pancreas produces insulin, but the body does not utilize the insulin correctly. This is primarily due to peripheral tissues insulin resistance where insulin receptor or other intermediates in the insulin signaling pathways within body cells are insensitive to insulin and consequently glucose does not enter the tissues leading to hyperglycemia or elevated blood-glucose concentrations (Albright, 1997). Obesity, which generally results in

impaired insulin action, is the common risk factor for this type of diabetes and most patients with diabetes type II are obese (Nolte and Karam, 2001) and will ultimately require multi antidiabetic agents to maintain adequate glycemic control (Gerich, 2001). There are many ways in treatment of diabetes type II patients such as 1- increase physical activity to reduce weight (consequently insulin resistance). 2- Reduce intake of dietary fat and adequate intake of complex carbohydrates and fiber, which improves insulin action and secretion through medical intervention, i.e. several commercial pharmaceutical that either enhances insulin action or secretion. The first treatment option is most beneficial as drug therapy may be associated with the long term underlying and undesired effects such as excessive weight loss (Jain and Saraf., 2008).

Mode of classification	Type I Diabetes Mellitus	Type II Diabetes Mellitus
Ages at onset	Juvenile –onset (JOD) occurs predominantly in children and young adults	Maturity Onset Diabetes (MOD) occurs predominantly in middle –aged or old people
Insulin dependence	Insulin dependent diabetes (IDDM). A patient reqires insulin therapy to prevent ketoacidosis	Non insulin dependent diabetes (NIDDM) give insulin treatment for better control often advisable especially in younger patients.

Table 2.1. Classification of Diabetes Mellitus (Jain and Saraf., 2008).

- Type III gestational diabetes, this type of diabetes occurs during pregnancy. During pregnancy the need for insulin appear to increase and gestational diabetes occurs at the late stages of pregnancy. This type of diabetes mellitus may goes away once the baby has been born but type II diabetes may develop later in life, in women who has gestational diabetes.

2.1.1. Factors influencing the prevalence of diabetes mellitus

There are many factors influence the prevalence of diabetes such as age, sex, obesity, lifestyle and gender.

2.1.2. Symptoms and diagnosis of diabetes mellitus

- Intense hunger and thirst.
- Frequent urination.
- Unusual weight loss.
- Increased fatigue.
- Itchy skin Slow healing of cuts and wounds.
- Frequent infections.
- Blurred vision.
- Numbness or tingling, especially in your feet and hands.
- Sexual dysfunction among men.

2.2. Glucose metabolism

2.2.1. Digestion and absorption of carbohydrates

Like any machine the body requires fuels to provide energy for it to function. The fuels the body needs come from the food we eat, which are made up of carbohydrates (sugars and starch), proteins and fats. These carbohydrates, proteins and fats are split apart by digestive enzyme into their basic building blocks glucose, amino acids and fatty acids respectively.There is a series of chemical reactions in the metabolic pathway which produces energy from glucose and this pathway is called the Krebs cycle that is coupled to the electron transfer chain present in all cells (Krall and Besar., 1989).

There are three stages (Fig 2.1) that must occur in order to generate energy from the food:

- The first stage involves the breakdown of large molecules into smaller units, i.e. proteins hydrolyzed to their twenty kind of amino acids, polysaccharides are hydrolyzed to simple sugars (glucose and fructose) and fats hydrolyzed to fatty acids and glycerol by lipases enzymes.
- The second stage involves the small units from the first stage are degraded into a few simple units that play a pivotal role in the metabolism. Here most of the sugars, amino acids and fatty acids are converted into acetyl CoA and small amount of adenosine triphosphate (ATP) is generated.
- The third stage consists of citric acid cycle and oxidative phosphorylation, the final pathway in the oxidation of fuel molecules. Acetyl units are completely oxidized at this stage to CO_2 and four pairs of electrons are transferred to nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD) for each acetyl group that is oxidized. ATP is then generated as electrons flow from the reduced form of these carriers to O_2 in a process known as oxidative phosphorylation. The bulk of ATP is generated in this stage (Stryer and Jeremy, 2007).

2.2.2. Hormones Control of Carbohydrate Metabolism

Hormones such as insulin and glucagon play an important role in regulation of glucose metabolism and maintain the hemostats in the body. The proper functions of the body are dependent on precise control of the glucose concentration in the blood. The normal fasting level of glucose in the blood is 3.8-5 mmol/l. If the concentration of

glucose in blood is too high (above 8 mml/l) a condition known as hyperglycemia results. Hyperglycemia may temporarily exist as a result of eating a meal rich in carbohydrates.

If the concentration of glucose is too low (below 3.8 mml/l), a condition of hypoglycemia exists. Hypoglycemia is characterized by general weakness, trembling, drowsiness, headache, profuse perspiration, rapid heartbeat, and possible loss of consciousness. These definitions are illustrated in Figure 2.2.

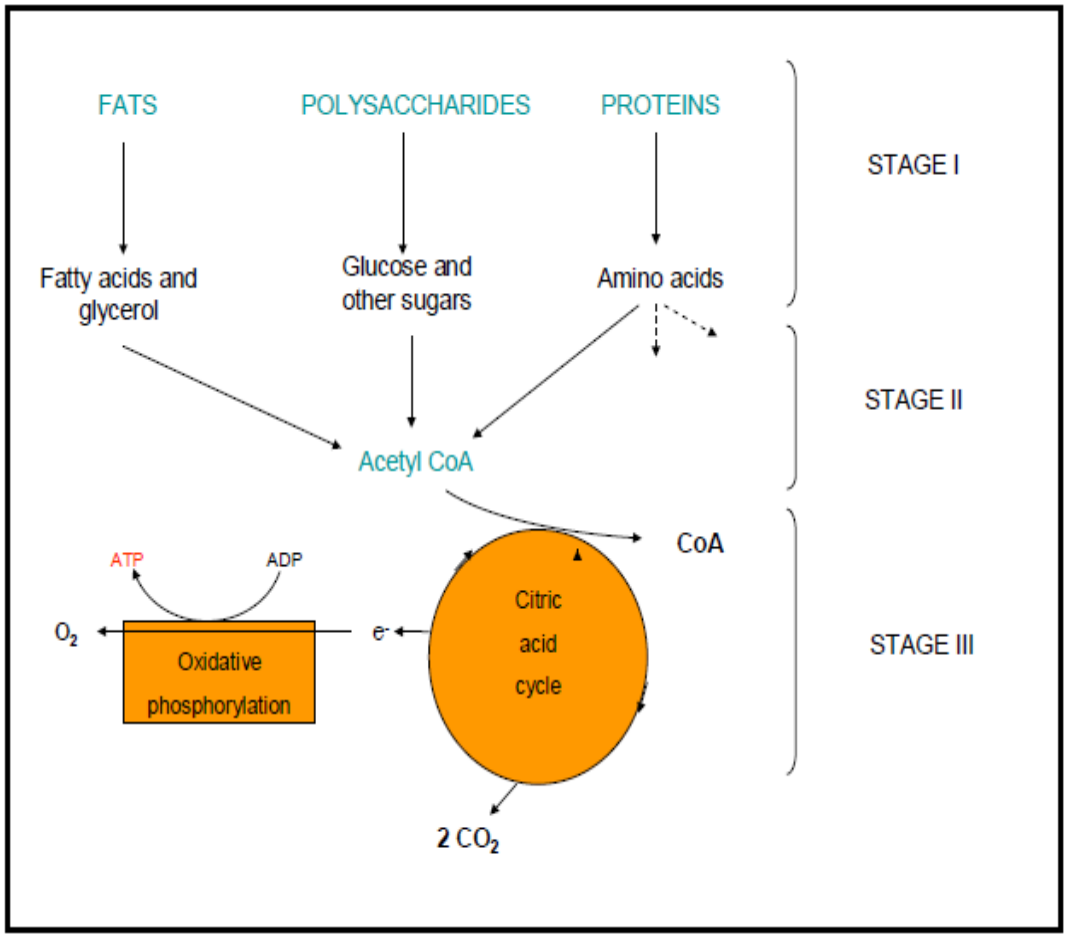

Figure 2.1. The extraction of energy from food . In the first stage large molecule in food broken down into smaller units, in stage two the small units are degraded to a few simple units that play a role in metabolism and the third stage consists of the citric acid and oxidative phosphorylation (Stryer and Jeremy, 2007).

2.2.3. Insulin

From section 2.2.2 it is noted that insulin plays an important role in the metabolism of glucose and any disruption to any stage of its metabolism will be reflected on the effectiveness of insulin in the body, which contributes to the growing symptoms of diabetes. To understand the disease, one needs to first understand the normal physiology on insulin action, which it plays important role in glucose hemostasis. Insulin is secreted in response to a rise in blood glucose concentrations and stimulation of the β cells of the pancreas by parasympathetic nervous system. Insulin stimulated anabolic processes and inhibits catabolic processes. It is the predominant hormone (along with glucagon) in the control of blood-glucose levels (figure 2.2). Insulin is a protein made up of long chains (A and B chain) of amino acids. The β-cells have the ability to take 86 amino acids and hook them together to form a long chain of amino acids called proinsulin. The active hormone exists as a dipeptide connected by two disulphide bond (Stryer and Jeremy, 2007)

After eating a meal rich in carbohydrates, the body starts to estimate mechanism for the digestion and absorption of carbohydrates. Hyperglycemia occurs, when the glucose rises to high levels.Then,the β cells of the pancreas secrete insulin to begin specific interrelated mechanisms aim to maintain the level of glucose in the body. The insulin has four major actions. These include:

- Increase the movement of glucose through membrane of adipose and muscle cells.
- Enzyme system starts multi-reactions in the liver and muscle cells for conversion of glucose to glycogen.
- Slow-down of gluconeogenesis and regulation of lipogenesis in liver and muscle cells.

Stimulation of general effect by promotion of protein synthesis and growth ((Klover and Mooner, 2004).

The aim of these actions is to promote the entrance of glucose and amino acids in cells of muscle tissues, adipose tissue and connective tissue. Glucose enters the cell by facilitated diffusion along an inward gradient created by low intracellular free glucose, and by the availability of a specific carrier called transporter. In the presence of insulin, the rate of movement of glucose into the cell is greatly stimulated in a selective fashion. During fasting, insulin concentrations are low while the counter-regulatory hormones glucagon, catecholamines and growth hormone promote hepatic glycogenlysis and gluconeogenesis from lactate, amino acids and glycerol (hydrolysis of triglyceride).

Metabolic actions of insulin result from its interaction with the insulin receptor (IR) found in all insulin responsive target cells (liver, muscle and adipose tissue). As shown in Figure 1.3 insulin binds to the α-subunit of insulin receptor (IR) and activates the intrinsic tyrosine kinase activity of the β-subunit of the receptor. Activated IR results in the subsequent phosphorylation of intracellular substrates including insulin receptor substrates (IRSs) such as IRS-1 and -2, phosphatidylinositol (PI) 3-kinase, and protein kinase B (PKB). Normal insulin action leads to increased glycogen synthesis, glucose transport, and lipogenesis, and decreased gluconeogenesis, glycogenolysis, and lipolysis (Klover and Mooner, 2004).

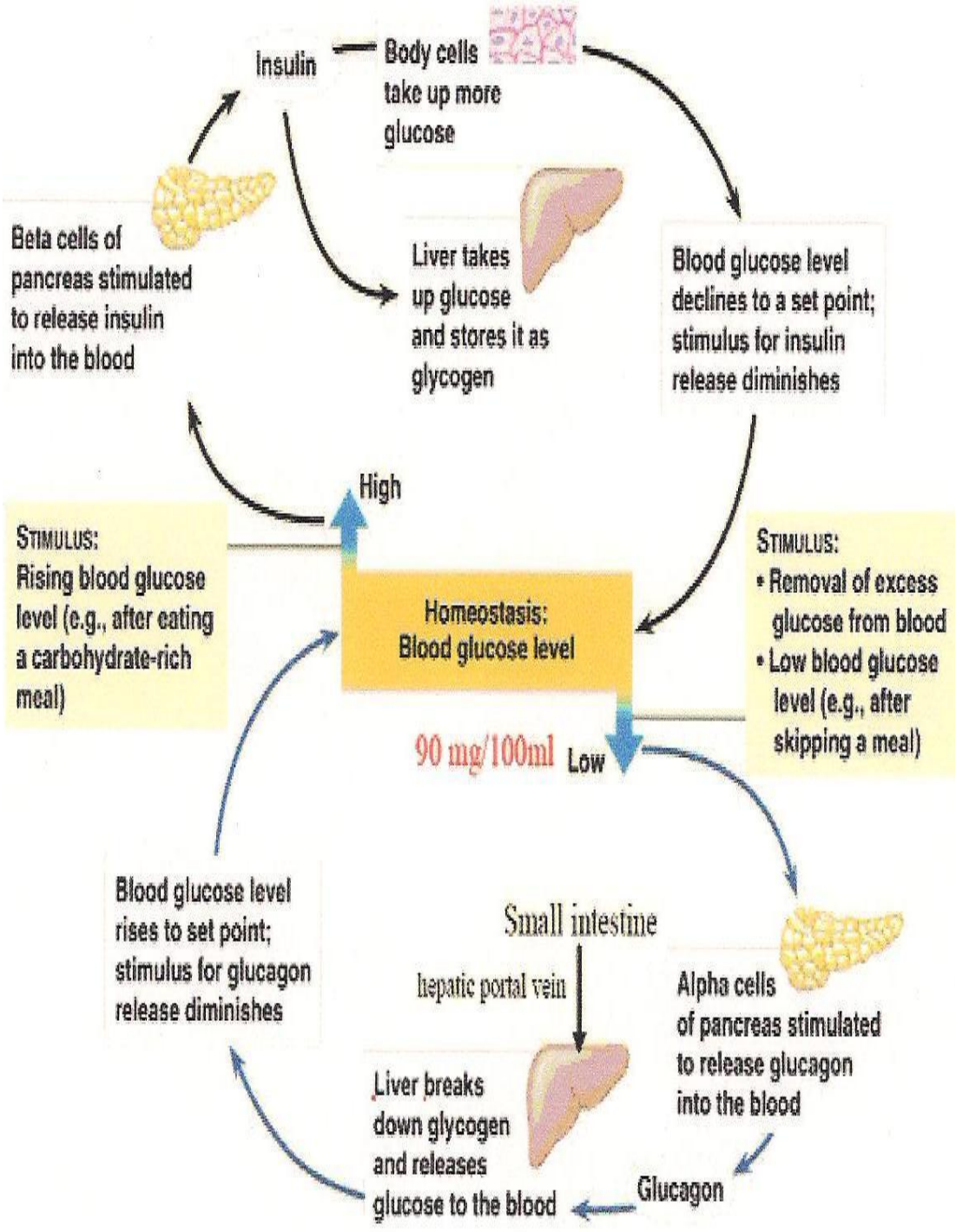

Figure 2.2. Hormonal control of glucose metabolism. When glucose level shows elevation (after a meal of rich carbohydrates), the insulin promotes the glucose metabolism. Glucagon promotes the glucose metabolism when reduction of glucose level occurs (Cheng and Fantus, 2005).

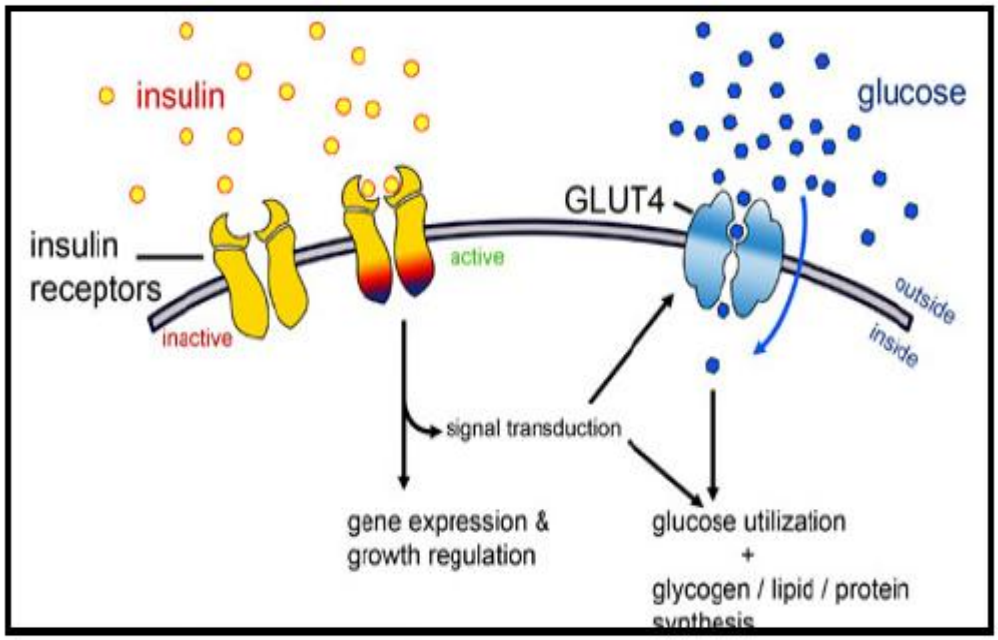

Figure 2.3. Insulin binding and activation of GLUT4 . The binding of insulin to insulin receptor stimulate the release of glucose which leads to glucose utilization in the cell (Klover and Mooner, 2004).

Glucose transporter type 4 (GLUT4) is a glucose transport protein found in fat and striated muscle cells. When a person with normal glucose metabolism ate the meal rich carbohydrates, insulin is secreted from the pancreas. Insulin starts to send signals to fat and muscles cells to absorb glucose from the blood by specific mechanism. Insulin invokes these cells to allow the insulin receptor binds on the surface of fat and muscle cells. Binding the insulin receptor will induce the GLUT4 protein to move from reserves held inside cells; GLUT4 can also be recruited to the cell surface through muscle contraction. In the absence of insulin or muscle contraction, GLUT4 is stored in vesicles within the cell. When GLUT4 is at the cell surface, glucose is transported into the cell down its concentration gradient. This process is called facilitated diffusion, which is very important in glucose metabolism because it create another process called phosphorylation. In this process, a phosphate group is then immediately added to the glucose by the enzyme hexokinase producing glucose-6-phosphate. These molecules can be used as an energy

source when it is metabolized through glycolysis and the Krebs cycle to produce energy units such as ATP and FADH.

Defects in GLUT4 activity have been implicated in some forms of insulin resistance or pre-diabetes **t**ype II. Insulin resistance is a condition where the pancreas secretes insulin, but fat, and muscle cells do not respond to it in taking up glucose. The pancreas compensates by secreting extra insulin to help cells take up glucose, and insulin-resistant patients often have high levels of glucose as well as a high blood insulin concentration. The pancreas eventually fails to keep up with the body's need for insulin, resulting diabetes type II.

2.3. Pathophysiological of diabetes mellitus type II

Diabetes mellitus type II is a complex metabolic disorder resulting from excessive hepatic glucose production, decreased pancreatic insulin secretion and insulin resistance in target tissues, mainly muscle and the liver (Ahmed and Goldstein., 2006). Diabetes mellitus type II is believed to be caused by both genetic and environmental factors. The most important risk factors for diabetes mellitus type II are believed to be obesity and oxidative stress. Initially, insulin resistance is compensated for, by increased insulin secretion by pancreatic β cell, but with time, these become exhausted and diabetes type II results (Cheng and Fantus, 2005). The pathogenesis of type II is summarized in figure 2.4. Initially, in the face of insulin resistance, compensatory increases in pancreatic insulin secretion are able to maintain normal glucose concentrations. However, as the disease progresses, insulin production gradually diminish, leading to progressive stages of hyperglycemia. Hyperglycemia is first exhibited in the postprandial state, since uptake by skeletal muscle is the metabolic fate of the majority of ingested carbohydrate energy, and then during fasting. As insulin secretion decreases, hepatic glucose production, normally

attenuated by insulin, increases. This increase is primarily responsible for the elevation of fasting glucose levels in patients with diabetes mellitus type II.

Elevation of blood glucose levels are thought to occur via a number of pathways, primarily via deleterious effects on pancreatic ß cell function, which together with insulin resistance is thought to play a critical role in the pathogenesis of type II diabetes. The adverse effects of hyperglycemia on ß cell dysfunction can be divided into three distinct phenomena: glucose desensitization, ß cell exhaustion, and glucose toxicity (Kilpatric and Robertson, 1998). Glucose desensitization is a physiological adaptation in which the pancreatic ß-cell becomes rapidly yet reversibly refractory to a short exposure of elevated glucose. ß-Cell exhaustion is the reversible depletion of the pool of intracellular insulin following long-term exposure to elevated glucose. It is thought that the ß-cell defects associated with these two phenomena are initially reversible but that they eventually become irreversible following prolonged exposure to elevated glucose, a process that is termed glucose toxicity. One mechanism by which the effects of glucose toxicity are thought to be mediated is oxidative stress, a term used to describe imbalance between levels of free radicals and antioxidants, so hyperglycaemia is known to be one of the main causes of oxidative stress in patients with type II diabetes (Robertson et al, 2004).

2.4. Complications of diabetes mellitus

- Diabetic retinopathy

  This is very common complication of diabetes mellitus which affects the retina and more commonly affect type I patients. Two types are common, the nonproliferative type where the blood vessels are closed off or weaken in the eye and this leads to blurred vision without blindness. The proliferative type causes a

proliferation or spouting of blood vessels in the retina which may lead to severe eye problem which may be resulted in cataract formation (Touchette, 2005).

- Diabetic nephropathy :

  The main changes in kidney of the diabetic occur within the glomerulus where cellular changes lead to a decrease in the surface area available to filter the blood and, consequently, a decrease in the glomerular filtration rate (GFR). In the people with this condition, the nephrons are unable to filter out impurities in the blood, and these begin to leak and impurities that supposed to be removed from the body end up re-circulating in the blood (Touchette, 2005).

- Diabetic neuropathy

  While diabetes mellitus doesn't impair the brain or spinal cord, it does effect the peripheral nerves in the rest of the body.This may be led to signal transduction errors, which are interpreted aberrantly (as pain in hands and feet ulceration, edema). Charcot arthropathy or muscle wastage (thigh, calf, trunk and hands), loss of autonomic function (postural hypotension, foot ulcers,and abnormal sweating), and marked weight loss (Williams and Pickup., 1999)

- Cardiovascular diseases

  Diabetes medication may cause certain chemical change in some of the substances found in the blood and this may lead to blood vessels narrowing or clogging up completely, resulting in a condition known as atherosclerosis. Hypertension is also a contributor of cardiovascular associated with diabetes mellitus (Touchette, 2005)

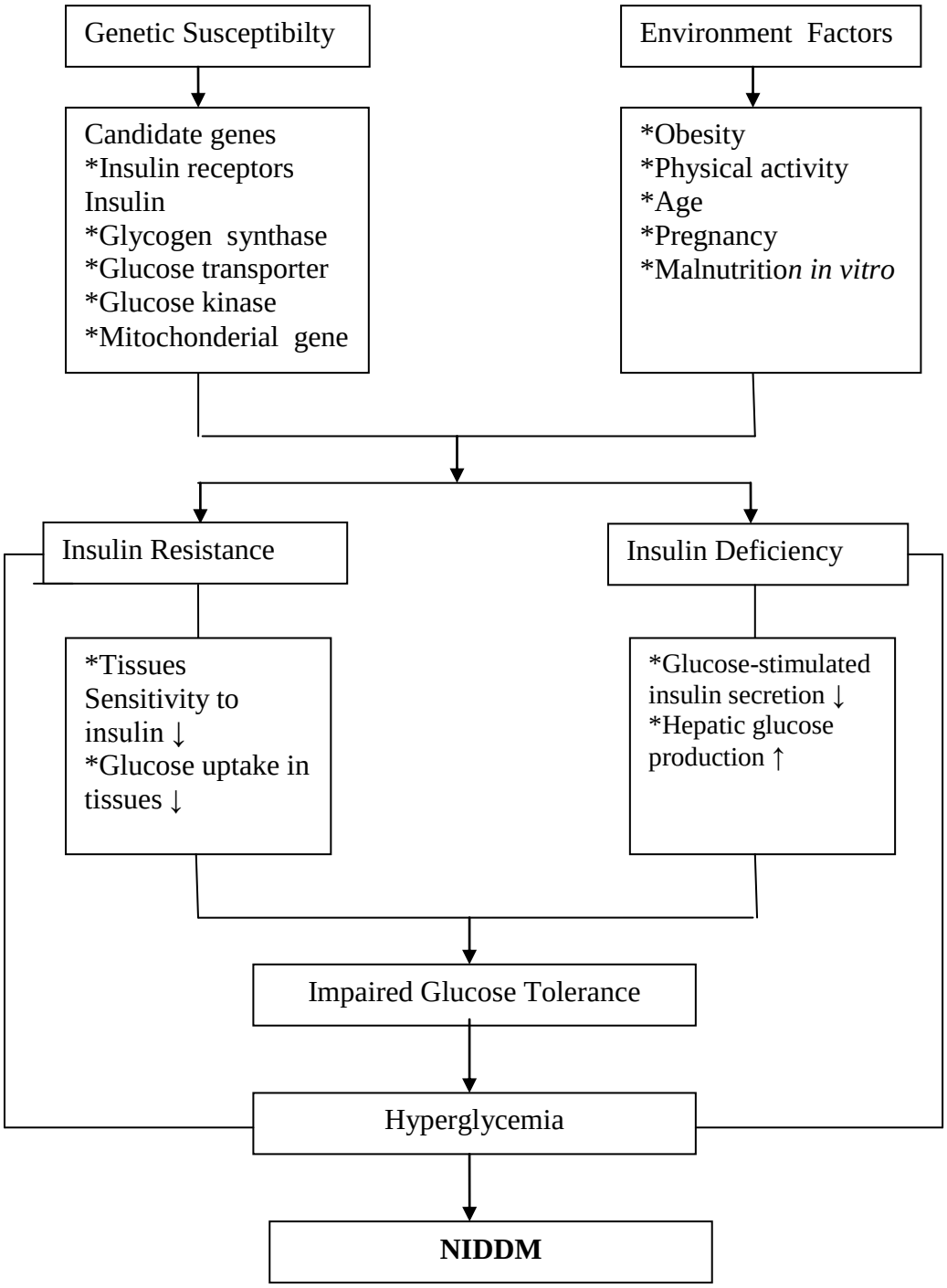

Figure 2.4. Progressive pathogenesis of type II diabetes mellitus (DeFronzo, 2004)
NIDDM = Non–insulin-dependent diabetes mellitus,

2.5. Treatment and management of type II diabetes mellitus

The goal of diabetes management is to keep blood-glucose hemostat as close to normal as safely possible. Since diabetes may be caused to risk for critical complications such as diabetes retinopathy, diabetes nephropathy and diabetes neuropathy measures to control blood pressure and cholesterol levels are an essential part of diabetes treatment as well. People with diabetes must take responsibility for their day to day care. Currently, diabetes type II is controlled and managed by a combination of diet restriction, weight reduction programs and oral hypoglycemic drugs (Day and Bailey, 2006; Evans and Rushakoff, 2007). Orally administered hypoglycemic agents (e.g. sulfonylureas, repaglinide, metformin, α-glucosidase inhibitors, thiazolidinediones (TZD) and dipeptidyl peptidase IV (DPP-IV) are used first (either alone or in combinations of different classes) together with dietary restriction and exercise programs (Day and Bailey, 2006). When hyperglycemia becomes severe, patients are usually switched to insulin injections. However, current anti-diabetic medications have toxic side effects, including, but not limited to, nausea, diarrhea, hypoglycemia, inflammation of the nasal passages and throat, upper respiratory tract infections, urinary tract infection, headache, liver problems, lactic acidosis and weight gain (Bastaki, 2005; Evanst al., 2007). Despite the intensive use of current anti-diabetic agents . Nathan (1993) reported that many (more than 50%) diabetic patients still exhibit poor glycemic control and some (18%) develop serious complications within six years of diagnosis. This study shows the need for seeking of new active antidiabetic agents from other sources. Figure 2.5 shows the summary of management of diabetes mellitus type II.

2.6. Mechanism of action of conventional oral hypoglycaemic drugs

Oral hypoglycemia agents exert their glucose lowering effects via a variety of mechanisms (Figure 2.6). These mechanisms of action include: reduction of hepatic glucose production, (e.g. metformin, a biguanide), enhancement of insulin secretion by pancreatic beta cells, (insulin secretagoges) improvement of insulin sensitivity (e.g. TZDs and metformin) and inhibition of intestinal glucose digestion and absorption (α glucosidase inhibitors). The use of these drugs is however, limited by the fact that they have adverse side effects, such as potential hypoglycaemia (e.g. sulfonylurea), weight gain, (e.g. meglitinides, sulfonylurea and thiazolidinesdiones), gastro-intestinal discomforts (α-glucosidase inhibitors and α-amylase inhibitors) and lactoacidosis (metformin) (Cheng and Fantus, 2005).

2.6.1. Oral antidiabetic agents

Five categories of oral antidiabetic agents are available namely; insulin scretagogues , biguanides , thiazolidinediones , α- glucosidase inhibitors and dipeptidyl peptidase IV (DPP- IV) inhibitors

2.6.1.1. Insulin secretagogues : sulfonylureas

Sulfonylurea (SU) drugs have been available in the United States since 1954. Second-generation SUs (glyburide, glipizide, and glimepiride) are more potent and probably safer than first-generation SUs (chlorpropamide, tolbutamide, acetohexamide, and tolazamide) but essentially of equal efficacy. The SUs bind to the SU receptor, found on the surface of pancreatic beta cells. This interaction leads to a closure of voltage-dependent potassium adenosinetriphosphate (KATP) channels, facilitating cell membrane depolarization, calcium entry into the cell, and insulin secretion. Thus, SUs allow for

insulin release at lower glucose thresholds than normal. The main side effects of sulfonylureas are weight gain. Given that these drugs directly stimulate insulin secretion from pancreatic β-cells irrespective of plasma glucose levels, the risk of hypoglycemia is associated with all sulfonylureas (Cheng and Fantus, 2005).

2.6.1.2. Biguanides

Biguanides (e.g: Metformin) are a relative old antidiabetic drug that improves the peripheral insulin effect at the musculature and inhibits gluconeogenesis by enhancing the take of glucose into the peripheral cells . The most side effects of metformin are hepatic impairment , renal impairment, congestive heart failure, metabolic acidosis and dehydration (Silvio and Inzucchi, 2002).

2.6.1.3. Insulin sensitizers (thiazolidinediones)

The thiazolidinediones (TZD) currently are available in many forms: Rosiglitazone, Pioglitazoneand and Troglitazone. Troglitazon was introduced in the United States.and it was later removed from the market because of rare idiosyncratic hepatocellular injury (Murphy et al, 2000). The most prominent effect of TZDs is increased insulin-stimulated glucose uptake by skeletal muscle cells. Thus, these agents decrease insulin resistance in peripheral tissues, then hepatic glucose production is decreased. Thiazolidinediones enhance the responsiveness and efficiency of beta cells, presumably by decreasing glucose and free fatty acid levels, both of which have deleterious effects on insulin secretion. (Silvio and Inzucchi, 2002). The major side effects of rosiglitazone and pioglitazone are weight gain, edema, anemia, pulmonary edema and congestive heart failure.

2.6.1.4. α-Glucosidase inhibitors

Acarbose is a novel antidiabetic drug that attenuates postprandial hyperglycaemia by delaying carbohydrates digestion. Acarbose exerts its inhibitory effects on the glucosidases, a family of membrane bound enzymes in the intestine that are involved in the digestion and uptake carbohydrate into the blood. Acarbose initiates a cascade of events which leads improved metabolic control in type II diabetes mellitus, by stimulating both the synthesis and secretion of insulin and in addition improves glycemic control when administrated concurrently with other antidiabetic agents. The main side effects of α-glucosidase inhibitors are on gastrointestinal tract. Specifically, bloating, abdominal discomfort, diarrhea and flatulence occur in about 20% of patients. Also α-glucosidase inhibitors are contraindicated in patients with irritable bowel syndrome or severe kidney or liver dysfunction. Inflammatory bowel disease is a relative contraindication (Hanefeld, 1998).

2.6.1.5. Dipeptidyl peptidase IV (DPP- IV) inhibitors

Treatment of diabetic patients with drugs from the incretin family is one of the basic and central treatment tools available to the clinician today. The first dipeptidyl peptidase IV (DPP-IV) inhibitor, Sitagliptin (brand name Januvia) and saxagliptin (Onglyza) were approved by the US Drug Administration in 2006 and 2007 respectively as treatment for diabetes concurrently with lifestyle changes (Karagiannis et al, 2012). The hormone glucagon-like peptide-1 or GLP-1 plays an important role in glucose homeostasis in the body. It has a profound action on both enhancing the pancreas cells to stimulate the insulin and delaying stomach emptying.Unfortunately, it is active only for a very short time because it is broken down by an enzyme called dipeptidyl peptidase-4, or DPP-IVinhibitor. The main function of the DPP-IV such as stagliptin and saxagliptin is

based on inhibition the enzyme activity which causes prolonging the effect of GLP-1, and hence enhances insulin secretion and the slowed emptying of the stomach. Most of the diabetic patients have impaired GLP-1 secretion and elevated DPP-4 activity. Since DPP-4 inhibitors enhance the body's own ability to release insulin, they can only be used in the treatment of diabetes type II. As the synthetic drugs, the DPP-IV inhibitors have side effects may be appeared in the diabetic patient.such as inflammation of the nasal passages and throat, upper respiratory tract infections, urinary tract infection and headache (Karagiannis et al, 2012).

2.7. Hypoglycemic studies

According to the American Association for Diabetes, two tests either the fasting blood glucose or the glucose tolerance test (GTT) should be done at different times in order to diagnose diabetes mellitus. The glucose tolerance test, GTT, was designed originally to determine the tolerance for the glucose. "Tolerance" refers to the body's ability to handle (tolerate) glucose. However, the test is not that simple. The outcome of the test depends on a number of factors, including the ability of the intestine to absorb glucose, the capacity of the liver to take up and store glucose, the capacity of the pancreas to produce insulin, the amount of "active" insulin it produces, and the sensitivity of the cells in the body to the action of insulin. For the test, the subject fasts overnight and is then given a specific amount (100 grams) of glucose by mouth, during 2 hours, the glucose level return to below normal. Since the dose of glucose is taken by mouth, the test is called oral glucose tolerance test or sometimes the glucose is given subcutaneously and called subcutaneous glucose tolerance test (SGTT).

In diabetic patient, fasting blood-glucose level may rises above 6.2 mmol/l-7.8 mmol/l and falls back to control values only after 4-6 hours later or fail to fall to normal