CHAPTER II

LITERATURE REVIEW

Diabetes Mellitus

Diabetes mellitus is defined by the American Diabetes Association (ADA) Expert Committee in their 1997 recommendations as a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidney, nerves, heart and blood vessels. Thus, diabetes covers a wide range of heterogeneous diseases.

The diagnostic criteria and the classification of diabetes were firstly based on the report of the World Health Organization (WHO) in 1965, then by National Diabetes Data Group (NDDG) in 1979, and followed by simplified recommendations by WHO in 1980. There were slightly changed in a new publication by the WHO in 1985. The last recommendations have been published by ADA in 1997 and by the WHO in 1999. The recommendation by ADA in 1997[1] proposed that the fasting plasma glucose concentration be used for screening and diagnosing diabetic patients. The WHO in 1999 recommended an oral glucose tolerance test for diagnosis when casual plasma glucose is in the certain range, between 5.5 and 11.1 mmol/L. Definition of glucose tolerance states according to the ADA 1997 and the WHO 1999 criteria is shown in table 1.

Table 1: Definition of glucose tolerance states according to the ADA 1997 and the WHO 1999 criteria [5].

		2-h plasma, mmol/L (mg/dL)		
		<7.8 (140)	7.8-11.0 (140-199)	≥11.1 (200)
Fasting plasma glucose, mmol/L (mg/dL)	<6.1 (110)	Normal	IGT	Diabetes on an isolated 2-h hyperglycemia. IPH (isolated postchallenge hyperglycemia)
	6.1-6.9 (110-125)	IFG	IFG and IGT	
	>7.0 (126)	Diabetes on an isolated fasting hyperglycemia		Diabetes on both fasting and 2-h hyperglycemia

IFG=impaired fasting glucose; IGT=impaired glucose tolerance; IPH=isolated postchallenge hyperglycemia

There are various forms of diabetes; most commonly broken down into 2 types: type 1 and type 2. Type 1 diabetes (T1D) most commonly diagnosed during childhood, formally known as juvenile-onset diabetes. The common cause of T1D is about the immune system destruction of pancreatic beta cells, which are responsible for making insulin, the hormone that regulates blood glucose. [26]

T1D accounts for 5% - 10% of all diagnosed diabetes cases, according to the National Institute of Diabetes and Digestive and Kidney Disease (NIDDK).Type 2 diabetes (T2D), formally known as non-insulin-dependent or maturity-onset diabetes, is the commonest form of the disease. T2D accounts for 85-95% of all cases worldwide and affects 5-7% of the world's population [27]. T2D is characterized by disorders of both insulin action and insulin secretion. The global prevalence of T2D will double between 1995 and 2025, to 270 million people. The greatest increases will be in the developing would, among economically productive adults aged 45-65 years. T2D affects approximately 17 million individuals in the United States. In particular, ethnic minorities, such as African Americans and Hispanic Americans, are affected disproportionately, having a 2-fold to 3-fold higher prevalence of disease compared with age-matched Caucasian patients.

T2D is a heterogeneous syndrome due to the interaction of various environmental factors with multiple diabetogenic genes, which cause various combinations of insulin resistance and beta cell failure. Both defects are partly genetically and environmentally determined and both are exacerbated by hyperglycemia.

The aims of the treatment of T2D are to ameliorate the symptoms of hyperglycemia, and to prevent early death and the acute and long-term tissue complication. Complications in T2D are related to glycemic control. Hyperglycemia has acute effects on well-being and will cause polyuria, thirst and polydipsia, as well as exposing a diabetic patient to the risk of intercurrent infections. In the longer term, chronic hyperglycemic increases the risk of tissue complications of micro- and macroangiopathy.

Side effects of medicine for diabetes mellitus

There are many drugs that are used in treatment of T2D. The main classes of oral agents are shown in table 2.[27-28]

Although growing number of oral pharmacologic options are now available for the treatment of T2D, the effectiveness of such therapies still remains inadequate and have some side effects upon using for a period of time.

Metformin is a biguanide that lowers blood glucose by several mechanisms retailed to increase insulin sensitivity, mainly decreasing hepatic glucose output, but also increasing peripheral glucose uptake. It does not cause weight gain but may promote weight loss. Major side effects are nausea, anorexia or diarrhea, which occur in about one-third of patients. A rare but serious side effect is lactic acidosis, with high mortality.

Sulphonylureas stimulate insulin secretion by binding to receptors on the $\beta-$ cell plasma-cell membrane, leading to closure of the adenosine triphosphate (ATP) - sensitive K channel, membrane depolarization, opening of calcium channels, calcium influx into the cell and exocytosis of insulin granules. They are only effective if there is residual b-cell function. The most serious side effect is hypoglycemic, which is most likely with glibenclamide and long-acting preparations such as chlorpropamiside in the elderly and alcohol intake patients.

Meglitinides, such as repaglinide and nateglinide, are analogues of the non-sulphonylurea portion of glibenclamide. They stimulate insulin secretion by binding near the sulphonylurea receptor/ ATP-sensitive K^{+} channels, and are used to control postprandial hyperglycemia. They can also be used in conjunction with metformin.

Thiazolidinediones, rosiglitazone, and pioglitazone are insulin sensitizers that bind to the nuclear peroxisome proliferation-active receptor$-\gamma$ (PPARγ) and enhance the expression of certain insulin-sensitive genes. Their use is associated with weight gain and fluid retention, and they should not be used in combination with insulin or in those who has a history of cardiac failure or renal insufficiency.

Acarbose inhibits α—glucosidase enzymes in the gut, there by delaying carbohydrate absorption and reducing postprandial blood glucose peaks. Its use has been limited by gastrointestinal side effects such as flatulence, diarrhea and cramping.

Orlistat inhibits pancreatic lipase and reduces the absorption of fat from the gut by up to 30%. It can aid weight lose in obese patient, in conjunction with hypocaloric diet. Gastrointestinal side effects are relatively common and include flatulence, fatty stool, increased frequency of defecation and fecal incontinence.

Table2: Oral agents used in the treatment of type 2 diabetes [27-28]

Action	Class	Mechanism
Increase insulin secretion	Sulphonylureas	Blind to sulphonylurea receptor on β cell, leading to closure of ATP-sensitive potassium channels.
	Meglitinide analogues	Blind to sulphonylurea receptor on β cell, leading to closure of ATP-sensitive potassium channels
Reduce insulin resistance	Thiazolidinediones	PPARγ agonist
Reduce insulin resistance, reduce hepatic glucose output	Biguanides	Not known
Delay absorption of carbohydrate	α-Glucosidase inhibitors	Inhibits α—glucosidase Increase fiber in diet
Reduce weight	Agents that reduce fat absorption	Inhibits pancreatic lipase
	Centrally acting agents	Serotonin and norepinephrine reuptake inhibitor

ATP= adenosine triphosphate: PPARγ= peroxisome proliferators-activated receptor-γ

Complication of Diabetes

AGE accumulate within the various organs that are damaged in diabetes, with the accumulation rate of these AGE accelerated by hyperglycemia. The intermolecular collagen cross-linking caused by AGE leads to diminished arterial and myocardial compliance and increased vascular stiffness, phenomena that are considered to explain partly the increase in diastolic dysfunction and systolic hypertension seen in diabetic subjects. AGE accumulate in most sites of diabetes complications, including the kidney, retina, and atherosclerotic plaques [29-31]. AGE have been measured and reported to be linked to the sustained effects of prior glycemic control on the subsequent development of vascular complications.

In the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications trial [32]. subjects treated previously with intensive glucose control displayed decreased carotid intima-media thickness, many years after the levels of glycosylated hemoglobin (HbA_{1c}) between the intensively and conventionally treated groups became indistinguishable [33,34]. It has been postulated that these effects have occurred as a result of "hyperglycemic memory" and may involve AGE. [16-17]

Diabetic nephropathy

The kidney is a target for AGE-mediated damage and is also a contributor to circulating AGE concentrations as seen in settings such as diabetes because the kidney is the major site of clearance of AGE .

Animal studies have clearly demonstrated a pathogenic role for AGE and RAGE in diabetic nephropathy. Diabetic animals have significant increases in renal AGE[35]. and these abnormalities have been linked to various structural aspects of diabetic nephropathy, including glomerular basement membrane thickening, mesangial expansion, glomerulosclerosis, and tubulointerstitial fibrosis. [36] Administration of AGE albumin in murine models has resulted in changes similar to that observed in diabetic nephropathy, including glomerular basement membrane thickening, mesangial matrix expansion, and increased collagen IV and TGF-β expression . [37] The strongest evidence of a role for AGE in the development of diabetic nephropathy has come from

studies targeting the AGE-RAGE pathway. Specifically, renal pathological changes are reduced by AGE formation inhibitors such as aminoguanidine , (±)-2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanidilide (OPB-9195), and ALT-946, as well as with agents that are postulated to reduce AGE accumulation such as the putative cross-link breaker ALT-711, also known as alagebrium.[38] Treatments targeting RAGE such as sRAGE, which acts as an endogenous antagonist to the full-length receptor and a RAGE-specific neutralizing antibody, have also been shown to attenuate nephropathy.[39] Furthermore, RAGE knockout mice rendered diabetic have less renal functional and structural injury.[40-41]

Diabetic ocular disease

CML and other AGE have been localized to retinal blood vessels in patients with type 2 diabetes and found to correlate with the degree of retinopathy.[42] When nondiabetic animals are infused with preformed AGE albumin, the adducts accumulate around and within the pericytes, colocalize with AGE-Rs, induce basement membrane thickening, and contribute to the breakdown of the inner blood-retinal barrier.[43-44] Furthermore, retinal vascular endothelial cells exposed to AGE show abnormal endothelial nitric oxide synthase expression, which may account for some of the vasoregulatory abnormalities seen in the retinal microcirculation in diabetes.[45-46] *In vitro* studies have also demonstrated the up-regulation of VEGF in retinal cells after exposure to AGE, potentially promoting retinal neovascularization and increasing permeability to proteins across the retinal barrier.[47-48]

Diabetic peripheral neuropathy

Elevated levels of AGE have been documented in the peripheral nerves of subjects with diabetes. The AGE-RAGE axis is a likely mechanism linking microangiopathy and neuropathy, and is supported by the colocalization of CML, RAGE, NF-κB, and IL-6 to epineurial vessels, perineurium and endoneurial vessels. It has been demonstrated in murine models that AGE worsen diabetic neuropathy by reducing sensorimotor conduction velocity and decreasing blood flow to peripheral nerves.[49]

Administration of AGE and subsequent RAGE activation replicates the effects of hyperglycemia, whereas RAGE antibody administration suppresses both NF-κB activation and expression of IL-6 transcripts in sciatic nerve studies. [50] A series of experiments on RAGE-null mice have demonstrated that CML and AGE effects are not seen in the dorsal root ganglia of such animals, and neither is diabetes-induced NF-κB activation. [51] Interestingly, despite inactivation of RAGE (through deletion of RAGE gene or blockade with sRAGE or receptor antibodies), the activation of NF-κB and expression of IL-6 mRNA still occurred at a low level. The RAGE-null status also did not protect from the diabetes-induced loss of PGP9.5-positive small fibers. This confirms that AGE-RAGE has a substantial role in mechanisms leading to neuropathy, especially sensory deficits, but is unlikely to be the sole factor responsible for progressive neurological damage in diabetes.

Atherosclerotic disease

AGE are likely to be linked to atherosclerosis in multiple ways, including enhancing endothelial dysfunction, elevating vascular low-density lipoprotein (LDL) levels by reducing LDL uptake, promoting plaque destabilization via effects on matrix metalloproteinases, inducing neointimal proliferation, and inhibiting vascular repair in response to injury. [52] Serum levels of AGE have increased in patients with type 2 diabetes and coronary heart disease. [53-54] Furthermore, AGE have been localized to atherosclerotic lesions, fatty streaks, lipid-containing smooth muscle cells, and macrophages in individuals with diabetes. [55] A correlation between tissue AGE concentration and the severity of atherosclerotic lesions has also been demonstrated. There appears to be multiple potential mechanisms whereby AGE may enhance atherosclerosis. AGE quench nitric oxide and impair LDL removal by trapping LDL in the subendothelium and decreasing LDL receptor recognition of AGE-modified LDL. AGE binding to apolipoprotein (apo) B impairs its hepatic clearance, and induces increased retention of LDL in the aortic wall and increased recognition by macrophages at this site [56]. Consequently, there is increased localization of AGE-LDL in vessels and increased production of foam cells, accelerating atheroma formation.

Endothelial migration of monocytes, one of the first steps in atherogenesis, is dependent upon up-regulation of vascular cell adhesion molecule (VCAM)-1 expression, and AGE have increased VCAM-1 expression via activation of the key nuclear transcription factor NF-، B.[57] Forbes *et al.* have shown that attenuation of the plaque area can be achieved in a murine model of diabetes-associated atherosclerosis with the AGE inhibitor aminoguanidine as well as with the putative AGE cross-link breaker alagebrium[58,59]. These two disparate pharmacological interventions resulted in reduced accumulation of AGE within the aortas, reduced expression of RAGE, and diminished expression of prosclerotic growth factors and various collagens. [60] Animal studies using sRAGE, either as a preventative strategy or delayed intervention, resulted in suppressed vascular lesion formation. [61]

Diabetic cardiomyopathy and peripheral arterial disease (PAD)

Patients with diabetes are more prone to develop cardiomyopathy and heart failure than nondiabetic subjects. Furthermore, cross-linking of collagen is considered to play a role in the development of diabetic cardiomyopathy and PAD. A positive correlation between serum levels of AGE and the isovolumetric relaxation time, a parameter measured on echocardiography reflecting cardiac function, has been documented in patients with type 1 diabetes. [62] High serum levels of the fluorescent AGE pentosidine are also associated with both increased carotid intima media wall thickness and arterial stiffening. [63] Increased levels of pentosidine and malondialdehyde (an indicator of lipid peroxidation) have also been observed in diabetic patients with PAD. [64]

Hypoglycemic effect of *Gymnema sylvestre*

Gymnema sylvestre (GS) is one of the Asclepiad strains that grows in South-east Asia. Their therapeutic effects for treating diabetes mellitus, rheumatic arthritis and gout have been well known for a long time. The administration of the dried leaf powder of GS regulated the blood sugar levels in alloxan diabetic rabbits. GS therapy not only produced blood glucose homeostasis but also increased the activities

of the enzymes affording the utilization of glucose by insulin dependent pathways. It controlled phosphorylase levels, gluconeogenic enzymes and sorbitol dehydrogenase. The uptake and incorporation of [^{14}C] glucose into the glycogen and protein are increased in liver, kidney and muscle in GS administered diabetic animals when compared to the untreated ones. Pathological changes initiated in the liver during the hyperglycemic phase are reversed by controlling hyperglycemia by GS.[18]

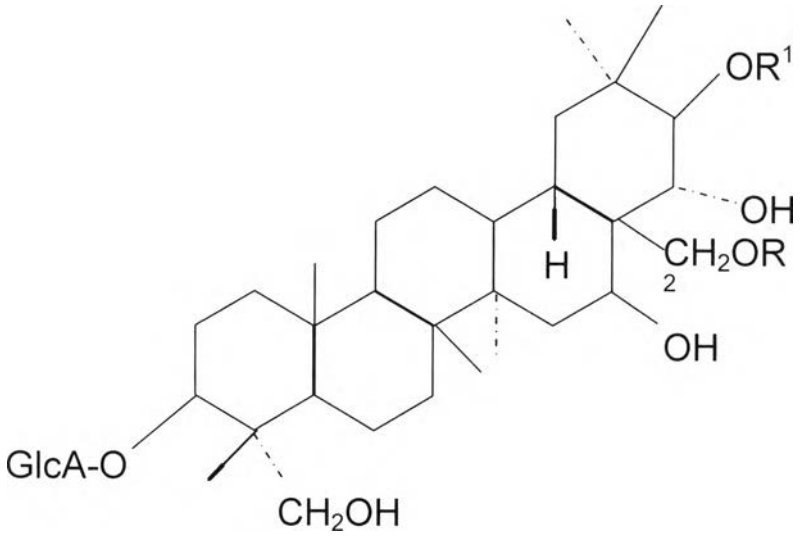
Gymnema sylvestre (GS) leaves extract was administered (400 mg/day) to 27 patients with T1D on insulin therapy. Insulin requirements came down together with fasting blood glucose and glycosylated hemoglobin (HbA1c) and glycelated protein levels. While serum lipids returned to near normal levels with GS therapy. Glycosylated hemoglobin and glycosylated plasma protein levels remained higher than controls. T1D patients on insulin therapy only showed no significant reduction in serum lipids, HbA1c or glycosylated plasma proteins when followed up after 10-12 months. GS therapy appears to enhance endogenous insulin, possibly by regeneration or revitalisation of the residual beta cells in T1D. [19]

The effectiveness of GS leaves extract in controlling hyperglycemia was investigated in 22 T2D patients on conventional oral anti-hyperglycemic agents. GS (400 mg/day) was administered for 18-20 months as a supplement to the conventional oral drugs. During GS supplementation, the patients showed a significant reduction in blood glucose, glycosylated hemoglobin and glycosylated plasma proteins, and conventional drug dosage could be decreased. Five of the 22 diabetic patients were able to discontinue their conventional drug and maintain their blood glucose homeostasis with GS alone. The data suggested that the beta cell may be regenerated or repaired in T2D patients on GS supplementation. This is supported by the appearance of raised insulin levels in the serum of patients after GS supplementation.[20]

Investigation of hypoglycemic activity of major saponin constituents from gymnemic acid, a crude saponin fraction of GS, exposed six triterpene glycosides, gymnemosides a,b,c,d,e, and f and nine gymnemic acids. [21] The inhibitory activity of each triterpene glycoside from gymnemic acid was examined to determine its impact on the increase of serum glucose level in oral glucose-loaded rats. Gymnemoside b and gymnemic acids III, V, and Vii were found to exhibit a little inhibitory activity against

absorption, but the principal constituents, gymnemic acid I and gymnemasaponin V, lacked this activity.[22]

Gymnemic acid



	R^1	R^2
Gymnemic acid I	Tigloy	Ac
Gymnemic acid II	2-metylbutyr	Ac
Gymnemic acid III	2-methybutyr	H
Gymnemic acid IV	Tigloy	H

Figure 1 Basic molecular structure of gymnemic acid from *Gymnema sylvertre*

Study on an alcoholic extract of GS on insulin secretion from rat islets of Langerhans and several pancreatic beta cell lines shows that Gs can stimulate insulin release from HIT-T15, MIN6 and RINm5F beta cells and from islets in the absence of any other stimulus. GS-stimulated insulin secretion was inhibited in the presence of 1 mM EGTA. Blockage of voltage-operated Ca^{2+} channels with 10 μM israpidine did not significantly affect GS-induced secretion, and insulin release in response to GS was independent of incubation temperature. Examination of islet and beta cell integrity after exposure to GS, by trypan blue exclusion, indicated that concentrations of GS that stimulated insulin secretion also caused increased uptake by dye. Two gymnemic acid-enriched fractions of GS, obtained by size exclusion and silica gel chromatography, also caused trypan blue uptake. There results confirm the stimulatory effects of GS on insulin release, and indicate that GS acts by increasing cell permeability, rather than by stimulating exocytosis by regulated pathways.[23]

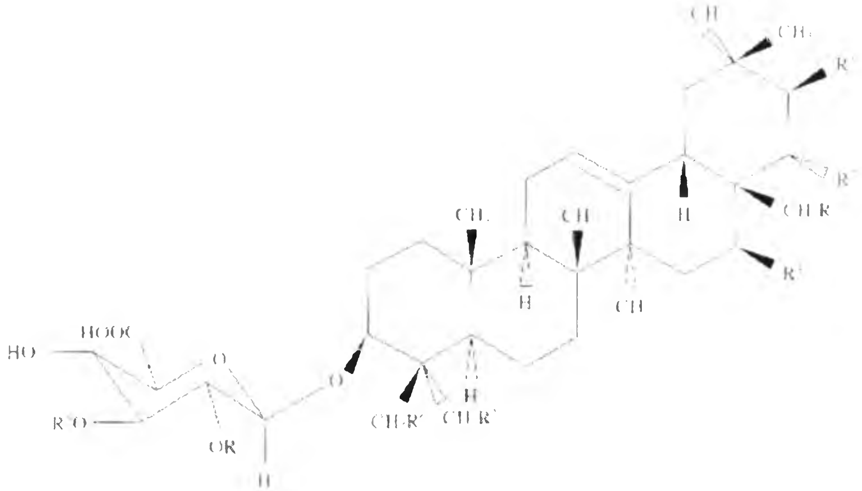
Investigators at the Toyama Medical and Pharmaceutical University in Sugitani, Japan, concluded that gymnemic acid IV from an extract of *Gymnema sylvestre* may be an anti-obesity and hypoglycemic ingredient. Their research indicated that gymnemic acid IV reduced blood glucose levels in diabetic mice six hours after administration, but it did not affect blood glucose levels of normal mice. Researchers concluded that the insulin-releasing action of gymnemic acid may contribute to the antihyperglycemic effect by the leaves of gymnema.[24]

Hypoglycemic effect of *Gymnema inodorum*

The problem of GS is its property in suppressing sweetness and tastes bitter. *Gymnema inodorum* (GI) has advantages that it neither suppresses sweetness nor has bitter taste. The leaves of GI have been known to be effective for some diseases including diabetes mellitus, rheumatic arthritis and gout. The crude saponin mixtures extracted from GI leaves inhibited glucose absorption in the isolated intestinal tract and suppressed the increased blood glucose in rats [65]. In one study, they examined the relationship between chemical structure and pharmacological activity of the four components from GI leave extracts (GiA-1, GiA-2, GiA-5 and GiA-7). These components

were the derivatives of (3β,4α,16β)-16,23,28-trihydroxyolean-12-en-3-yl-β-D-glucopyranosiduroic acid. GiA-2, GiA-5 and GiA-7 have suppressive effects on the high K^+-induced contraction, an increase in Δ PD and the increased blood glucose level in the glucose tolerance test have –H at the 21st position and $-CH_2OH$ at 4β of aglycon. On the other hand, GiA-1 that does not have any effects on the three parameters mentioned above has –H at the 21st position and $-CH_3$ at 4β of aglycon. It is suggested that the inhibitory effect of triterpenoids in *Gymnema* leaves on glucose absorption from the intestinal tract relies on $-CH_2OH$ at 4β.[66]

Saponin fractions (F-I to F-IV) from GI leaves extract were obtained by high-performance liquid chromatography and their effects of glucose availability were studied on a high K -induced contraction of guinea-pig intestinal smooth muscle, O_2 consumption on guinea-pig ileum, glucose-evoked transmural potential difference (ΔPD) of guinea-pig everted intestine and blood glucose level in glucose tolerance tests on rats. The extracts of GI leaves suppressed the intestinal smooth muscle contraction, decreased the O_2 consumption, inhibited the glucose evoked-transmural potential, and prevented high blood glucose level. The study suggests that the component of GI inhibits the increase in the blood glucose level by interfering with the intestinal glucose absorption process.[66]



Component	R	R^2	R^3	R^4	R^5	R^6	R	R^8
GiA 1	H	H	OH	OH	H	H	β-Glc	H
GiA 2	H	H	OH	OH	OH	H	[illegible]	H
GiA 5	H	O-NMA	OH	OH	OH	H	H	β-Glc
GiA 7	H	O-NMA	OH	OH	OH	H	H	H

β-Glc = β-glucopyranosyl; O-NMA = O-[illegible]

Figure 2 Structure formula of triterpenoids in *Gymnema inodorum* [27]

Advanced glycation endproducts and the progress of diabetic vascular complication

Diabetic vascular complication is a leading cause of acquired blindness, end-stage renal failure, a variety of neuropathies and accelerated atherosclerosis, which could account for disabilities and high mortality rates in patients with diabetes. Although several hyperglycemia- elicited metabolic and hemodynamic derangements have been implicated in the pathogenesis of diabetic vascular complication, the process of formation and accumulation of advanced glycation end products (AGE) and their mode of action are most compatible with the theory 'hyperglycemic memory'. Further, there is a growing body of evidence that AGE and their receptor (RAGE) axis is involved in the pathogenesis of diabetic vascular complication. Indeed, the engagement of RAGE with AGE is shown to elicit oxidative stress generation and subsequently evoke inflammatory responses in various types of cells, thus playing an important role in the development and progression of diabetic micro- and macroangiopathy. These observations suggest that down-regulation of RAGE expression or blockade of the RAGE downstream signaling may be a promising target for therapeutic intervention in diabetic vascular complication.

AGE are a complex group of compounds formed via a nonenzymatic reaction between reducing sugars and amine residues on proteins, lipids, or nucleic acids. The major AGE *in vivo* appear to be formed from highly reactive intermediate carbonyl groups, known as α-dicarbonyls or oxoaldehydes, including 3-deoxyglucosone, glyoxal, and methylglyoxal.[67,68] Some of the best chemically characterized AGE in humans include pentosidine and N(carboxymethyl)lysine (CML). Some AGE like pentosidine have intrinsic fluorescence, and as such, tissue and plasma fluorescence can be used as markers of AGE accumulation. Other AGE such as CML are nonfluorescent and may be detected by approaches such as ELISA.

Apart from endogenously formed products, AGE can also originate from exogenous sources such as tobacco smoke and diet.[69-72] Food processing, especially prolonged heating, has an accelerating effect in the generation of glyco-oxidation and lipo-oxidation products, and a significant proportion of ingested AGE is absorbed with

food. Tissue and circulating AGE levels are higher in smokers and in patients on high AGE diets, with concurrent increases in inflammatory markers. [69-71] Furthermore, there is evidence from animal studies that exposure to high levels of exogenous AGE contributes to renal and vascular complications. [72-73] Nevertheless, it remains to be determined as to the relative importance of these exogenous sources of AGE in the pathogenesis of diabetic complications.

AGE often accumulate intracellularly [74] as a result of their generation from glucose-derived dicarbonyl precursors. [67] It is likely that these intracellular AGE play important roles as stimuli for activating intracellular signaling pathways as well as modifying the function of intracellular proteins. [67-75]

In diabetes, AGE accumulation may result from chronic hyperglycemia promoting the generation of AGE, and also with concomitant impaired renal function because the kidney is the major site of AGE clearance. AGE modified proteins may be more resistant to enzymatic degradation, [75] and it is likely that this further promotes local tissue AGE accumulation.

The effects of AGE may be classified as receptor-independent or -dependent, and can act intracellularly or circulate and act on cell surface receptors such as the receptor for AGE (RAGE). Advanced glycation occurs over a prolonged period, affecting long-lived proteins. The structural components of the connective tissue matrix and, in particular, basement membrane components such as type IV collagen are prime targets, but other long-lived proteins can also undergo advanced glycation, including myelin, tubulin, plasminogen activator 1, and fibrinogen. [76]

Extracellular matrix (ECM) proteins are susceptible to AGE modification because of their slow turnover rate. The formation of intermolecular and intramolecular cross-links with collagen as a result of the glycation process leads to structural alterations, leading to increased stiffness and resistance to proteolytic digestion. For example, AGE cross-linking on type I collagen and elastin leads to increased stiffness of blood vessels. [77-78] The composition of ECM is also modified by AGE, with increased

expression of ECM proteins, including fibronectin, types III, IV, and VI collagen and laminin, possibly mediated through up-regulation of key profibrotic cytokines such as TGF-β [79,80] and connective tissue growth factor. [81]

Polymorphism of RAGE and diabetes

The role of RAGE in vascular disease, especially in diabetes, made it as a candidate gene for study to identify allelic variants and their role in RAGE pathogenesis. The gene of RAGE is located on chromosome 6 in the major histocompatibility complex (MHC), a region of the genome containing a number of inflammatory genes and the most dense region of genes in the genome. The RAGE gene is composed of 11 exons and a 3/UTR region, and within these exons a common variant in exon 3 (Gly82Ser) and 3 rare coding changes (Thr187Pro, Gly329Arg, Arg389Gln) exist. [82,83]

Due to the observed up-regulation of RAGE expression in disease, genetic variation within key transcriptional regions of RAGE might impact on vascular disease. Screening of this region for allelic variants identified numerous polymorphisms, and the two common single nucleotide polymorphisms are -374 T/A and -429T/C. Further investigation of transcriptional mechanisms revealed monocytic nuclear extract gave a loss of transcription factor binding site to the -374 A allele.[83] These data suggested these polymorphisms might affect a repressor mechanism leading to up-regulation of RAGE transcription.

The association between the -429 T/C and -374 T/A of RAGE gene polymorphisms and the complication of DM seems to be ethnic dependent. In the study of Hudson et al.,[84] they found the positive association between -429 C allele and diabetic retinopathy in T2D Caucasians. On the other hand, the study of dos Santos et al. found no association between the -429 C and -374A of the RAGE gene and diabetic retinopathy, diabetic nephropathy and ischemic heart disease in African-Brazilians with T2D.[10] Xu et al. found no association between -429 T/C and -374 T/A polymorphism of RAGE promoters with retinopathy in T2D patients in China.[87] Petrovic et al. also found

no association between -429 T/C and -374 T/A gene polymorphism of RAGE and coronary heart disease in Slovene population with T2D.[88] However, a study of Pettersson-Fernholm carried out on patients with type 1 diabetes has found a decreased risk of having proteinuria and cardiovascular disease among the AA homozygote compared to patients with TT or TA genotype of -374 T/A polymorphism. [89]