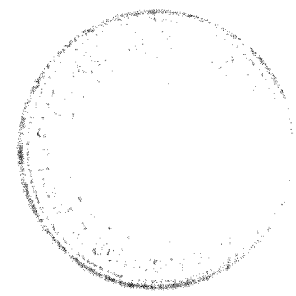

REVIEW OF LITERATURE

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The approach of modern medicine of treating the symptoms as against the holistic approach of traditional systems of medicine has lured the patient towards the safety and alternative modalities of treatments of the latter.

There is growing focus on the medicinal plants in the traditional health care system (Ayurveda, Unani, Homeopathy, Yoga etc) in solving health care problems. Plant based medicines are safe, effective, with less or almost no side effects.

These represent substantial portion of the global market. The WHO has appreciated traditional medicines and has evolved guidelines to support member states in their efforts to formulate remedies on traditional medicines and to study their potential usefulness, safety and efficacy (WHO, 1978).

India has more than 45,000 different plant species. Out of these only about 7,000 plants are used in Ayurveda, 600 in Siddha, 700 in Unani and 30 in modern medicines. There is an urgent need to explore the rich heritage of India in the medicinal plants. India can play a major role in international herbal market. Various medicinal plants have been screened for several biological activities. Screening of a plant for a specific biological activity generally depends upon its use in folk and tribal medicines. Herbal medicines (defined as preparations derived from plants and fungi, for example by alcoholic extraction or decoction, used to prevent and treat diseases) are an essential part of traditional medicine in almost any culture (Vickers A, Zollman CE 1999). In industrialized countries herbal drugs and supplements are an important market. Some countries like Germany have a long tradition in the use of herbal preparations marketed as drugs and figures for prescriptions and sales are stable (Schwabe, U 1999). In the US and the UK herbal medicinal products are marketed as "food supplements" or "botanical medicines". In recent years sales of such products have been increasing strongly in these countries (Brevoort P 1998; Barnes, J 2000). In the Third World herbs are mainly used by traditional healers (Bodeker GC 1996). According to the world health organization herbal medicines are used by 80% of Africans and large sections of world populations rely on it as their primary form of health care. Up to 25% of all prescription in Europe and America contain plant products (Patil et al., 2005).

Cucurbitaceae is a plant family commonly known as gourds or cucurbits and includes crops like cucumbers, squashes, luffas, and melons. The family is predominately distributed around the tropics, where those with edible fruits were amongst the earliest cultivated plants in both the Old and New Worlds. Most of the plants in this family are annual vines with large showy-blossoms, usually white or various shades of bright yellow to orange-yellow. The plants produce male and female flowers separately on the same plant, and the female flowers have inferior ovaries. The fruit is a kind of berry called a Pepo. There are about 118 genera in Cucurbitaceae, including 825 species (David Bates et al., 1990)

Momordica is a genus of about 45 species of annual or perennial climbing herbaceous plants belonging to the family Cucurbitaceae, natives of tropical Africa and southern Asia. Some species produce floral oils and are visited by specialist pollinators in the apid group. *Momordica* are grown in cultivation as ornamental plants, grown for their fleshy fruit, which are oblong to cylindrical in shape, orange to red in colour, prickly or warted externally, and burst when ripe, generally with elastic force, into irregular valves.

Momordica of Cucurbitaceae family has the following medicinally useful species (Kirtikar R etal., 1935, Nadkarni AK etal., 1982). *Momordica Charantia, Momordica balsamia, Momordica dioica, Momordica cochinchinesis, Momordica Schimperiana, Momordica subangulata blume, Momordica denudate, Momordica macrophylla gage, Momordica mixata, Momordica monodelpha, Momordica umbellate.*

Momordica cymbalaria Fenzl (Family: Cucurbitaceae) is tuberous perenninal with very slender scandent, branched, striate, pubescent or subglabrous stem, the whole plant is acrid, and the ovoid tuberous roots are reported to have been used for inducing abortion, a decoction being administered for this purpose. The extracts and the dried form of fruit and leaves were shown to have antidiabetic and hypolipidemic properties (Kameswararao B etal., 1999, 2000, 2003). Ethanolic extract is reported to have antiovulatory and abortifacient activity (Koneri R etal 2006)

Momordica cymbalaria:

Botanical synonyms	: Momordica tuberosa Cogn.
	: Luffa tuberosa Roxb.
Family	: Cucurbitaceae
Vernacular names	
Marathi	: Kadavanchi.
Kannada	: Karchi.
Tamil	: Atha laikai.
Malayalam	: Kattu paval.

The plant is a tuberous perennial with very slender scandent stem and found in western parts of India from Satara district in the north down to Tinnevelly in the south.

Leaves are Orbicular-reniform, glabrous, or with few scattered hairs, punctate (but not scarbid) on both the surfaces, deeply cordate at the base, obtusely but not deeply 5-7 lobed, the lobes short, acute or obtuse. Tendrils is Filiform, slightly pubescent, simple and Calyx hairy, tube short, broadly campanulate, narrowed at the base; lobes 6mm. Long lanceolate, acute. Corolla Pale yellow; segments obovate, obtuse, long, stamens2, filaments very short, thick, flattend; anthers, 2mm long, one two partite, the others three partite, the cells conduplicate, the connective board. Fruit Long broadly ovoid, slightly compressed, strophiolate, not margined, testa polished and shining.

Momordica dioica

It is dried ripe fruits of *Momordica dioica* belonging to family *Curcubitaceae* *Momordica dioica* is a species found in coastal Karnataka and Andhra Pradesh state.

Botanical synonyms: Momordica *dioica* Roxb. ex Willd.(Ali A etal.,1998,Michel H1995)

Family	: Cucurbitaceae
Vernacular names	
English	: Spine gourd.
French	: Margose de Ceylan
Hindi	: Kaksa.
Tamil	: Tumpai, Palupakkai
Malayalam	: Tumba karawila.

Roots of *Momordica cymbalaria*

***Momordica dioica* Roxb.ex Willd – Fruit**

Phytoconstituents:

Aliphatic constituents: 6-methyl tritriacont-50on-28-of and 8-methyl hentracont-3-ene. Sterol: Pleuchiol.; Pentacylic triterpene: Momodicaursenol, an unknown isolated from the seeds, has been identified as urs-12, 18(19)-dien-3 beta-ol. (Ali A etal.1998).

Pharmacological activity:

The fruits have been reported to possess antihyperlipidemic activity (Ali A etal.1998) and for the treatment of diabetes mellitus. Chloroform, ethyl acetate & ethanolic extract of *Momordica dioica roxb* fruit is previously reported to have the antidiabetic activity in alloxan induced experimental rats (Reddy GT etal., 2005), flavanoidal fraction from ethanolic extract of the fruit is reported to have hepatoprotective property (Kushwaha SK etal.,2005), hexane extract of the fruit is reported to have antifeedant property(Narasimhan S etal 2005), seed oil has shown insecticide property(Mishra D etal., 2006), ethanolic and aqueous extract of the root is reported to have antifertility activity(Shreedhar CS etal 2001). Further three triterpenes and two steroidal compounds were isolated from the dry root of *Momordica dioica* and have shown anticancer property (Luo L et.al 1998).

Insulin and glucose homeostasis

The important target tissues for regulation of glucose homeostasis by insulin are liver, muscle and fat; insulin also exerts potent regulatory effects on other cell types as well (Gilman AG et al. 1996). The specific stimulus for insulin secretion involves elevations in circulating levels of glucose. Stimulation of glucoreceptors in β-cell membrane activates an adenyl cyclase system and causes an influx of calcium ions. This altered intra-cellular ionic balance stimulates a contraction of a sub-cellular microtubule-microfilament system, which is involved in the transport and fusion of insulin containing secretary granules with the cell membrane. Fusion of the granule and cell membrane permits the granule content to be released. The cell membrane of islet cells contains five coupled systems (Craig CR et al., 1982). These are the substrate carriers, a receptor transducer complex, a calcium gate that controls Ca^{2+} entry, an adenyl cyclase system and the secretory complex. These systems are involved in specific stimulus for insulin secretion. Insulin catalyses the synthesis of

various macromolecules and prevents undue breakdown of proteins, carbohydrates and fat. Thus it promotes cell growth by deposition of carbohydrates, lipids and proteins. The most clearly measurable action of insulin is to facilitate the penetration of amino acids and simple sugars through the cell membrane of skeletal and heart muscle which otherwise have low permeability to these substances. Receptor sites on cell surface membrane bind insulin and this reaction leads to changes both in cell permeability and the activity of enzyme system within the cell. Thus reduction of blood glucose is brought about by:

1. Increasing glycogenesis
2. Increasing entry of glucose into insulin sensitive cells such as myocytes, hepatocytes and adipocytes
3. Inhibiting breakdown of lipids.
4. Increasing the rate of protein synthesis
5. Stimulating some cell ion transport mechanisms such as Na^+ / K^+ / ATPase
6. Decreasing gluconeogenesis

Insulin interacts with the target cells by binding to insulin receptor which is composed of two glycoprotein subunits, α and β. In the mature $\alpha_2\beta_2$ receptor, the extra cellular α-subunit confers high affinity insulin binding, whereas transmembrane β-subunit is responsible for transducing the signal of insulin binding to the interior of the cell. The cytoplasmic portion of the β-subunit bears strong homology to other transmembrane receptors with tyrosine kinase activity.

Receptor bound insulin triggers a cascade of intracellular responses. Due to the stimulation of tyrosine kinase activity some compounds in the β-subunit are phosphorylated (auto phosphorylation).

This leads to phosphorylation of some enzymes in the cytosol. Activation of these enzymes causes movement of transport proteins (glucose transporters) leading to the pharmacological action (Rang HP et al., 1999).

Relationship of insulin with glycogen, triglycerides and protein (Gilman AG etal., 1996): Insulin stimulates glucose storage in the liver as glycogen and in adipose tissue as triglycerides and amino acid storage in muscle as protein; it also promotes utilization of glucose in muscle for energy. Insulin inhibits the breakdown of triglycerides, glycogen, and the conversion of amino acids to glucose (gluconeogenesis).These

pathways are increased during fasting and in diabetic states. The conversion of amino acids to glucose and of glucose to fatty acids occurs primarily in the liver.

Metabolic Dearrangements in diabetic conditions (Vasudevan DM etal 2005; Rang HP etal., 1999; Lehniger 2001)**:** The changes are mainly due to the result of a low insulin-glucagon ratio.

Derangements in carbohydrate metabolism: Insulin deficiency decreases the uptake of glucose by cells. High glucagons levels decrease hepatic fructose-2, 6-bisphosphate level, there by decreasing the utilization of glucose. The insulin dependent enzymes are also less active. Net effect is an inhibition of glycolysis and stimulation of gluconeogenesis leading to hyperglycemia

Derangements in lipid metabolism: In mammals cholesterol synthesis is regulated by the intracellular cholesterol concentration by the hormones insulin & glucagon. The rate limiting step in the pathway of cholesterol synthesis is the conversion of HMG CoA into mevalonate. HMG coA is also hormonally regulated. Glucagon stimulates phosphorylation (inactivation), and insulin promotes dephosphorylation (activation). In diabetic conditions the purpose of fatty acids breakdown is to meet the energy requirements, this would lead to high free fatty acids in the plasma. There is a resultant hyperlipidemia especially an increase in non esterified fatty acids triglycerides and cholesterol. During diabetic conditions HMG CoA reductase enzyme activity is increased resulting in more cholesterol synthesis.

Derangements in liver glycogen synthesis: In normal conditions insulin inhibits hepatic glucose production. Decreases gluconeogenesis and glycogenolysis.

With insulin deficiency or insulin resistance, there is an increase in hepatic glucose production, decrease in the pheripheral glucose uptake and decrease in the conversion of glucose to glycogen in liver; this is mainly due to less availability of active form of enzyme glycogen synthetase which is responsible for the incorporation of glucose moieties into preexisting glycogen chain.

Pathogenesis of Type-2 diabetes mellitus (James MC et al 2001):

The two metabolic defects that characterize type-2 diabetes mellitus are:

1. A derangement in β-cell secretion of insulin
2. A decrease response of peripheral tissue to respond to insulin (Insulin resistance)

Patients with Type-2 diabetes may have an elevated, normal or low level of circulating insulin, depending on the chronicity of their disease, and have a relative lack of effective insulin. In patients with Type-2 disease, insulin and glucose levels usually are adequate to prevent ketoacidosis development. However, glucose can accumulate in the blood and can reach extremely high levels (more than 400 mg/dL), resulting in nonketotic hyperosmolar syndrome (NKHS).

1. *Deranged β-cell secretion of insulin:* A modest hyperinsulinemia may be observed, attributed to β-cell hyperresponsiveness to physiologic elevations in blood glucose, with the development of overt disease. The pattern of insulin secretion exhibits a subtle change. Early in the course of Type-2 diabetes, insulin secretion appears to be normal and plasma insulin levels are not reduced. However, the normal pulsatile oscillating pattern of insulin secretion is lost and the rapid first phase of insulin secretion triggered by glucose is obtunded. Collectively, these and other observations suggest derangements in β-cell response to hyperglycemia early in Type-2 diabetes, rather than deficiencies in insulin synthesis per se. Later in the cause of Type-2 diabetes a mild to moderate deficiency of insulin develops which is less severe than that of Type-1.

2. Insulin Resistance: An insulin resistance is a common pathologic state in which target cells fail to respond to ordinary levels of circulating insulin. It is frequently associated with a number of diseases, including chronic infection, human obesity and Type-2 diabetes (Donnelly R 2005). At the molecular level, impaired insulin signaling results from mutations or post translation modification of the insulin receptor itself or any of its down-stream effector's molecules. Insulin resistance could be accounted for by a defect in insulin binding to its receptor, there may be a decrease in the number of insulin receptor and more important, post receptor signaling by insulin is impaired. The binding of insulin to its receptor leads to translocation of glucose transporter (GLUT's) to the cell membrane, which in turn facilitates cellular uptake of glucose. Insulin Resistance is central to the clustering of multiple metabolic abnormalities and clinical syndromes. The clustering phenomenon was first described by Kylin in 1923 when he described the clustering of three clinical syndromes: hypertension, hyperglycemia, and hyperuricemia (Kylin in 1923).Yalow and colleagues in 1965 (Yalow RS et al., 1965) were first to discover an insulin assay and reported that IR

was a condition in which insulin does not produce the same glucose lowering effects seen in insulin-sensitive individuals. Dr. Reaven first described the metabolic and clinical associations of the many names of Syndrome X. In 1999, the World Health Organization had chosen a unifying definition for this syndrome of many names and elected to use the term metabolic syndrome rather than the insulin resistance syndrome (Alberti KGM etal., 1998)

Fructose and Insulin Resistance

Although fructose does not appear to acutely increase insulin levels, chronic exposure seems to indirectly cause hyperinsulinemia and obesity through other mechanisms. One proposed mechanism involves GLUT5, a fructose transporter that is found to have significantly higher expression levels in young Zucker obese rats compared to lean controls. As the rats age and become diabetic, GLUT5 abundance and activity is compromised, causing an even more marked insulin resistance over lean rats, implying a possible role of GLUT5 receptors in the pathology of metabolic syndrome associated with fructose feeding and insulin resistance (Litherland GJ etal 2004). In rats fed 66% fructose for 2 weeks, insulin receptor mRNA, and subsequent insulin receptor numbers in skeletal muscle and liver were significantly lower compared to rats fed a standard chow diet. Also, blood pressure and plasma TG increased in the fructose-fed rats, even though there was no change in plasma insulin, glucose, or body weight (Catena C etal 2003). Evidence shows these early steps in insulin signaling are important for insulin's metabolic effects. In a different study, it was found that after 28 days of fructose feeding there were no changes in insulin receptor concentration, but, insulin stimulated autophosphorylation, a mechanism necessary for insulin action, was reduced to 72% in the liver. Insulin receptor substrate (IRS) protein levels were similar, but there were significant decreases in insulin induced IRS (1/2) phosphorylation in both the liver and muscle of the fructose fed rats (Ueno M etal 2003). These changes are important, because it has been shown that the products of these insulin independent metabolic pathways lead to polyol formation and advanced glycation end products, which can contribute to the numerous complications and premature atherosclerosis seen in diabetic patients (Levine R. 1986). It is also known that such inflammations can lead to the pathogenesis of diabetes, and there is strong evidence suggesting that increased free fatty acids (FFA)

in diabetic subjects and fructose fed models play a role in the inflammatory state of insulin resistance. If FFA is not removed from tissues, as occurs in fructose fed insulin resistant models, there is an increased energy and FFA flux that leads to the increased secretion of TG. Insulin resistance has also been correlated with intracellular TG stores, which are involved in lipotoxicity and beta cell failure leading to diabetes (Ziegler O etal 2001). Another theory explaining how chronic fructose overnutrition can lead to type 2 diabetes is the hexosamine hypothesis, where hexosamine flux is thought to regulate glucose and satiety-sensing pathways. With overexpression of glutamine:fructose-6-phosphate amidotransferase, the key regulatory enzyme in hexosamine synthesis, the liver produces excess fatty acids, skeletal muscle becomes insulin resistant, and hyperinsulinemia results. This pathway of excess hexosamine flux leads to long-term storage of energy, and eventually obesity and type2 diabetes (McClain DA. 2002).

Oxidative stress

Oxidative stress is defined in general as excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Turko IV et al 2001; Maritim AC et al., 2003). ROS include free radicals such as superoxide ($^{\bullet}O_2^-$), hydroxyl ($^{\bullet}OH$), peroxyl ($^{\bullet}RO_2$), hydroperoxyl ($^{\bullet}HRO_2^-$) as well as nonradical species such as hydrogen peroxide (H_2O_2) and hydrochlorous acid (HOCl) (Turko IV et al 2001; Evans JL et al., 2002). RNS include free radicals like nitric oxide ($^{\bullet}NO$) and nitrogen dioxide ($^{\bullet}NO_2^-$), as well as nonradicals such as peroxynitrite($ONOO^-$), nitrous oxide (HNO_2) and alkyl peroxynitrates (RONOO) (Turko IV et al 2001; Evans JL et al ., 2002). Of these reactive molecules, $^{\bullet}O_2^-$, $^{\bullet}NO$ and $ONOO^-$ are the most widely studied species and play important roles in the diabetic cardiovascular complications.

$^{\bullet}NO$ is normally produced from L-arginine by endothelial nitric oxide synthase (eNOS) in the vasculature (Turko IV et al 2001). $^{\bullet}NO$ mediates endothelium-dependent vasorelaxation by its action on guanylate cyclase in vascular smooth muscle cells (VSMC), initiating a cascade that leads to vasorelaxation. $^{\bullet}NO$ also displays antiproliferative properties and inhibits platelet and leukocyte adhesion to vascular endothelium. Therefore, $^{\bullet}NO$ is considered a vasculoprotective molecule. However $^{\bullet}NO$ easily reacts with superoxide, generating the highly reactive molecule

$ONOO^-$, and triggering a cascade of harmful events (Turko IV et al 2001; Vega-Lopez S et al., 2004). Therefore its chemical environment, i.e. presence of $^{\bullet}O_2^-$, determines whether $^{\bullet}NO$ exerts protective or harmful effects.

Production of one ROS or RNS may lead to the production of others through radical chain reactions. $^{\bullet}O_2^-$ is produced by one electron reduction of oxygen by several different oxidases including NAD (P) H oxidase, xanthine oxidase, cyclooxygenase and even eNOS under certain conditions as well as by the mitochondrial electron transport chain during the course of normal oxidative phosphorylation, which is essential for generating ATP (Evans JL et al., 2003; Griendling KK & FitzGerald GA 2003; Griendling KK & FitzGerald GA 2003a; Taniyama Y & Griendling KK 2003). Under normal conditions, $^{\bullet}O_2^-$ is quickly eliminated by antioxidant defense mechanisms. $^{\bullet}O_2^-$ is dismutated to H_2O_2 by manganese superoxide dismutase (Mn-SOD) in the mitochondria and by copper (Cu)-SOD in the cytosol (Evans JL et al., 2003). H_2O_2 is converted to H_20 and O_2 by glutathione peroxidase (GSH-Px) or catalase in the mitochondria and lysosomes, respectively. H_2O_2 can also be converted to the highly reactive $^{\bullet}OH$ radical in the presence of transition elements like iron and copper.

While ROS are generated under physiological conditions and are involved to some extent as signaling molecules and defense mechanisms as seen in phagocytosis, neutrophil function, and shear-stress induced vasorelaxation, excess generation in oxidative stress has pathological consequences including damage to proteins, lipids and DNA.

ROS can stimulate oxidation of low-density lipoprotein (LDL), and ox-LDL, which is not recognized by the LDL receptor, can be taken up by scavenger receptors in macrophages leading to foam cell formation and atherosclerotic plaques (Boullier A et al., 2001). $^{\bullet}O_2^-$ can activate several damaging pathways in diabetes including accelerated formation of advanced glycation end products (AGE), polyol pathway, hexosamine pathway and PKC, all of which have been proven to be involved in micro- and macrovascular complications. $^{\bullet}O_2^-$ and H_2O_2 stimulate stress-related signaling mechanisms such as NF-κB, p38-MAPK and STAT-JAK resulting in VSMC migration and proliferation. In endothelial cells, H_2O_2 mediates apoptosis and pathological angiogenesis (Taniyama Y & Griendling KK 2003). Furthermore, $^{\bullet}O_2^-$

immediately reacts with $^{\bullet}NO$ generating cytotoxic $ONOO^-$ and this reaction itself has several consequences. First, $ONOO^-$ alters function of biomolecules by protein nitration as well as causing lipid peroxidation (Turko IV et al 2001). For example, potassium channels, which regulate the vasorelaxation response, are inhibited by nitration (Liu Y, Gutterman DD et al., 2002; Liu Y et al., 2002). As reviewed by Turko et al, increased levels of nitrotyrosine are associated with apoptosis of myocytes, endothelial cells and fibroblasts in diabetes (Turko IV et al 2001). Second, $ONOO^-$ causes single-strand DNA breakage which in turn activates nuclear enzyme poly (ADP-ribose) polymerase (PARP) (Soriano FG et al., 2001). Third, it decreases $^{\bullet}NO$ bioavailability causing impaired relaxation and inhibition of the antiproliferative effects of $^{\bullet}NO$ (Maritim AC et al., 2003). Furthermore, $ONOO^-$ oxidizes tetrahydrobiopterin (BH_4), an important cofactor for NOS, and causes uncoupling of NOS, which produces $^{\bullet}O_2^-$ instead of $^{\bullet}NO$ (Maritim AC et al., 2003). ROS-induced peroxidation of membrane lipids alters the structure and the fluidity of biological membranes, which ultimately affects function. All these pathological modifications contribute to the pathogenesis of vascular dysfunction.

Oxidative stress in diabetes

Direct evidence of oxidative stress in diabetes is based on studies that focused on the measurement of oxidative stress markers such as plasma and urinary F_2-isoprostane as well as plasma and tissue levels of nitrotyrosine and $^{\bullet}O_2^-$(Vega-Lopez S et al., 2004; Oberg BP et al., 2004). There are multiple sources of oxidative stress in diabetes including nonenzymatic, enzymatic and mitochondrial pathways.

Nonenzymatic sources of oxidative stress originate from the oxidative biochemistry of glucose. Hyperglycemia can directly cause increased ROS generation. Glucose can undergo autoxidation and generate $^{\bullet}OH$ radicals. In addition, glucose reacts with proteins in a nonenzymatic manner leading to the development of Amadori products followed by formation of AGEs. ROS is generated at multiple steps during this process. In hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also results in enhanced production of $^{\bullet}O_2^-$.

Enzymatic sources of augmented generation of reactive species in diabetes include NOS, NAD (P) H oxidase and xanthine oxidase (Guzik TJ et al., 2000;Guzik

TJ et al., 2002; Aliciguzel Y et al.,2003). All isoforms of NOS require five cofactors/prosthetic groups such as flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, BH_4 and Ca^{2+}-calmodulin. If NOS lacks its substrate L-arginine or one of its cofactors, NOS may produce $^{\bullet}O_2^-$ instead of $^{\bullet}NO$ and this is referred to as the uncoupled state of NOS(Maritim AC et al., 2003; Guzik TJ et al.,2000; Guzik TJ et al., 2002; Aliciguzel Y et al:,2003). NAD(P)H oxidase is a membrane associated enzyme that consists of five subunits and is a major source of $^{\bullet}O_2^-$ production(Guzik TJ et al.,2000; Guzik TJ et al., 2002; Kitada M et al., 2003; Etoh T et al., 2003). Guzik et al. investigated $^{\bullet}O_2^-$ levels in vascular specimens from diabetic patients and probed sources of $^{\bullet}O_2^-$ using inhibitors of NOS, NAD (P) H oxidase, xanthine oxidase and mitochondrial electron transport chain. This study demonstrated that there is enhanced production of $^{\bullet}O_2^-$ in diabetes and this is predominantly mediated by NAD (P) H oxidase. Furthermore, the NOS-mediated component is greater in patients with diabetes than in patients who do not have diabetes (Guzik TJ et al., 2002). It is also observed that NAD (P) H oxidase activity is significantly higher in vascular tissue (saphenous vein and internal mammary artery) obtained from diabetic patients (Ergul A et al., 2004). There is plausible evidence that PKC, which is stimulated in diabetes via multiple mechanisms, i.e. polyol pathway and Ang II, activates NAD (P) H oxidase (Amiri F et al., 2002).

The mitochondrial respiratory chain is another source of nonenzymatic generation of reactive species. During the oxidative phosphorylation process, electrons are transferred from electron carriers NADH and $FADH_2$, through four complexes in the inner mitochondrial membrane, to oxygen, generating ATP in the process (Green K et al., 2004). Under normal conditions, $^{\bullet}O_2^-$ is immediately eliminated by natural defense mechanisms. A study demonstrated that hyperglycemia-induced generation of $^{\bullet}O_2^-$ at the mitochondrial level is the initial trigger of vicious cycle of oxidative stress in diabetes (Nishikawa T et al., 2000; Brownlee M. 2001). When endothelial cells are exposed to hyperglycemia at the levels relevant to clinical diabetes, there is increased generation of ROS and especially $^{\bullet}O_2^-$, which precedes the activation of four major pathways involved in the development of diabetic complications. Nishikawa and colleagues demonstrated that generation of excess pyruvate via accelerated glycolysis under hyperglycemic conditions floods the mitochondria and causes $^{\bullet}O_2^-$ generation at the level of Complex II in the respiratory

chain. What is more important is that blockade of $^{\bullet}O_2^-$ radicals by three different approaches using either a small molecule uncoupler of mitochondrial oxidative phosphorylation (CCCP), over expression of uncoupling protein-1 (UCP1) or overexpression of Mn-SOD, prevented changes in NF-κB as well as polyol pathway, AGE formation and PKC activity. Based on this information, it has been postulated by several groups that mitochondrial $^{\bullet}O_2^-$ is the initiating snowball that turns oxidative stress into an avalanche in diabetes by stimulating more ROS and RNS production via downstream activation of NF-κB-mediated cytokine production, PKC and NAD(P)H oxidase. Thus, inhibition of intracellular free radical formation would provide a causal therapy approach in the prevention of oxidative stress and related vascular complications in diabetes.

Reactive species can be eliminated by a number of enzymatic and nonenzymatic antioxidant mechanisms. SOD immediately converts $^{\bullet}O_2^-$ to H_2O_2, which is then detoxified to water either by catalase in the lysosomes or by glutathione peroxidase in the mitochondria. Another enzyme that is important is glutathione reductase, which regenerates glutathione that is used as a hydrogen donor by glutathione peroxidase during the elimination of H_2O_2. Maritim and colleagues reviewed in detail that diabetes has multiple effects on the protein levels and activity of these enzymes, which further augment oxidative stress by causing a suppressed defense response (Maritim AC et al., 2003). For example, in the heart, which is an important target in diabetes and prone to diabetic cardiomyopathy leading to chronic heart failure, SOD and glutathione peroxidase expression as well as activity are decreased whereas catalase is increased in experimental models of diabetes(Maritim AC et al., 2003; Kaul N et al., 1996; Hayden MR & Tyagi SC 2003). In patients with chronic heart failure, all three enzymes are decreased in the smooth muscle (Linke A et al., 2005) and exercise training can upregulate the expression and activity of antioxidant enzymes. Increased isoprostane levels in diabetic patients with chronic heart failure are correlated with antioxidant status and disease severity (Cristina Polidori M et al., 2004). Thus, modulation of these enzymes in target organs prone to diabetic complications such as heart and kidney may prove beneficial in the prevention and management of heart failure and kidney failure.

Nonenzymatic antioxidants include vitamins A, C and E; glutathione; α-lipoic acid; carotenoids; trace elements like copper, zinc and selenium; coenzyme Q_{10} (CoQ_{10});

and cofactors like folic acid, uric acid, albumin, and vitamins B_1, B_2, B_6 and B_{12}. Alterations in the antioxidant defense system in diabetes have been reviewed (Vega-Lopez S et al., 2004). Glutathione (GSH) acts as a direct scavenger as well as a co-substrate for GSH peroxidase. It is a major intracellular redox tampon system. Vitamin E is a fat-soluble vitamin that prevents lipid peroxidation. It exists in 8 different forms, of which α-tocopherol is the most active form in humans. Hydroxyl radical reacts with tocopherol forming a stabilized phenolic radical which is reduced back to the phenol by ascorbate and NAD(P)H dependent reductase enzymes(Hensley K et al., 2000; Hensley K et al., 2004). CoQ_{10} is an endogenously synthesized compound that acts as an electron carrier in the Complex II of the mitochondrial electron transport chain. Brownlee et al reported that this is the site of $^{\bullet}O_2^-$ generation under hyperglycemic conditions (Nishikawa T et al., 2000; Brownlee M. 2001). CoQ_{10} is a lipid soluble antioxidant, and in higher concentrations, it scavenges $^{\bullet}O_2^-$ and improves endothelial dysfunction in diabetes(Hodgson JM et al., 2002; Hodgson JM, et al., 2003; Hodgson JM, et al., 2003). Vitamin C (ascorbic acid) increases NO production in endothelial cells by stabilizing NOS cofactor BH_4 (Heller R et al., 2001). α-Lipoic acid is a hydrophilic antioxidant and can therefore exert beneficial effects in both aqueous and lipid environments. α-lipoic acid is reduced to another active compound dihydrolipoate. Dihydrolipoate is able to regenerate other antioxidants such as vitamin C, vitamin E and reduced glutathione through redox cycling (Heller R et al., 2001).

Increased oxidative stress as measured by indices of lipid peroxidation and protein oxidation has been shown to be increased in both insulin dependent diabetes (IDDM), and non-insulin dependent (NIDDM) (Sato et al., 1979; Velazquez et al., 1991; Collier et al., 1992; MacRury et al., 1993; Neri et al., 1994; Yaqoob et al., 1994; Griesmacher et al., 1995; Niskanen et al., 1995; Laaksonen et al., 1996; Santini et al., 1997; Laaksonen and Sen, 2000; Cederberg et al., 2001), even in patients without complications. Increased oxidized low density lipo-protein (LDL) or susceptibility to oxidation has also been shown in diabetes (Collier et al., 1992; Neri et al., 1994; Yaqoob et al., 1994; Griesmacher et al., 1995; Laaksonen et al., 1996; Santini et al., 1997). Despite strong experimental evidence indicating that oxidative stress may determine the onset and progression of late-diabetes complications (Baynes, 1991; Van Dam et al., 1995; Giugliano et al., 1996), controversy exists about whether the increased oxidative stress is merely associative rather than causal in

DM. This is partly because measurement of oxidative stress is usually based on indirect and nonspecific measurement of products of reactive oxygen species, and partly because most clinical studies in DM patients have been cross-sectional (Laaksonen and Sen, 2000). The mechanisms behind the apparent increased oxidative stress in diabetes are not entirely clear. Accumulating evidence points to a number of interrelated mechanisms (Lyons, 1993; Cameron and Cotter, 1993; Tesfamariam, 1994; Cameron et al., 1996), increasing production of free radicals such as superoxide (Nath et al., 1984; Ceriello et al., 1991; Wolff et al., 1991; Dandona et al., 1996) or decreasing antioxidant status (Asayama et al., 1993; Tsai et al., 1994; Ceriello et al., 1997; Santini et al., 1997). These mechanisms include glycoxidation (Hunt et al., 1990; Wolff et al., 1991) and formation of advanced glycation products (AGE) (Lyons, 1993; Schleicher et al., 1997), activation of the polyol pathway (Cameron et al., 1996; Cameron and Cotter, 1993; Grunewald et al., 1993; Kashiwagi et al., 1994; De Mattia et al., 1994; Kashiwagi et al., 1996) and altered cell and glutathione redox status (Grunewald et al., 1993; Kashiwagi et al., 1994;1996; De Mattia et al., 1994) and ascorbate metabolism (Sinclair et al., 1991) antioxidant enzyme inactivation (Arai et al., 1987; Blakytny and Harding, 1992; Kawamura et al., 1992), and perturbations in nitric oxide and prostaglandin metabolism (Tesfamariam, 1994; Maejima et al., 2001).

Evidence from experimental models: A multitude of *in vivo* studies have been performed utilizing antioxidants in experimental diabetic models. The effects of antioxidants on oxidative stress are measured through certain observable biomarkers. These markers include the enzymatic activities of catalase, SOD, GSH-Px, and GSH-reductase, as well as thiobarbituric acid reactants (TBARS) levels, an indirect measurement of free-radical production that has been shown to be consistently elevated in diabetes. Normalization of the activity levels of any of these markers, and ultimately, the balance of free-radical production/removal, would be an effective method to reduce ROS-induced damage. Many animal studies have been completed with this aim in mind and indeed have shown that diabetes-induced alterations of oxidative stress indicators can be reversed when the animals are treated with various antioxidants.

Mekinova et al. demonstrated that supplementation of streptozotocin (STZ) diabetic rats with vitamins C, E, and beta-carotene for 8 weeks produced a significant reduction of TBARS levels, GHS, and GHS-Px, an increase in Cu-SOD, and no change in catalase activity in kidneys(Mekinova Det al.,1995). Treatment with vitamins C and E was also shown to decrease urinary albumin excretion, glomerular basement membrane thickness, and kidney weight in STZ diabetic rats (Kedziora-kornatowska K et al., 2003). In the same study, vitamins C and E significantly lowered malondialdehyde (TBARS) levels and GSH-Px activity while increasing catalase and SOD activities when compared to unsupplemented diabetic animals(Kedziora-kornatowska K et al., 2003). A study by Cinar et el. demonstrated that supplementation with vitamin E significantly lowered liver and lung TBARS levels and improved impaired endothelium-dependent vasorelaxation in STZ diabetic rat aorta (Cinar M et al 2001).

α-lipoic acid, which is involved in mitochondrial dehydrogenase reactions, has gained a considerable amount of attention as an antioxidant. Studies have demonstrated that intraperitoneal administration of α-lipoic acid to STZ diabetic Wistar rats normalizes TBARS level in plasma, retina, liver, and pancreas (Obrosova I et al., 2000). Obrosova et.al observed a reduction of GSH activity in diabetic retina and that supplementation with α-lipoic acid produced no change (Obrosova I et al., 2000). However, another study demonstrated an increase in aortic GSH-Px in STZ diabetic rats that was normalized by treatment with α-lipoic acid (Kocak G et al., 2000). Additionally, increased maximum contractile responses in diabetic aortic rings were ameliorated with α-lipoic acid treatment (Kocak G et al., 2000).

SOD activity is undoubtedly important to the regulation of oxidative status in diabetes. α-lipoic acid has been observed to normalize diabetes-induced decreases of SOD in rat heart (Maritim A et al., 2003) and retina(Obrosova I et al., 2000). One study demonstrated that treatment of STZ diabetic rats with α-lipoic acid reverses SOD-induced vasorelaxation, potentially due to the elimination of excess superoxide/hydrogen peroxide and the recovery of basal NO(Kocak G et al., 2000). A study by Brands et al. investigated the effect of oxidative stress in the development of hypertension in diabetes using the SOD mimetic tempol in a Type 1 model of diabetes where NOS is pharmacologically inhibited with a NOS inhibitor, L-NAME (Brands

MW et al., 2004). In this model, hyperglycemia causes hypertension implicating an important role for NO. Results of this study showed that if $^{\bullet}O_2^-$ is eliminated by tempol early in the disease process, the hypertension and decrease in glomerular filtration precipitated by diabetes are prevented.

Evidence from clinical trials: Although studies with antioxidants in experimental models as well as observational studies strongly suggest that antioxidants should confer beneficial effects in reducing cardiovascular complications in diabetes, clinical evidence for the use of antioxidants is not solid. It should be emphasized that clinical trials with antioxidants in diabetes are limited and majority of these trials focused on the use of vitamin E and C and lately α-lipoic acid.

Vitamin E demonstrated beneficial cardiovascular effects. In a double-blind, placebo-controlled, randomized study, vitamin E supplementation (1000 IU/day) for three months in patients with Type 1 diabetes (n = 41) significantly improved endothelium-dependent vasorelaxation(Skyrme-Jones RA et al., 2000). In another study, Beckman et al. reported that administration of vitamin E (800 IU/day) and C (1000 mg/day) combination for six months had a positive effect on endothelium-dependent vasorelaxation in Type 1 diabetic patients (n = 26)(Beckman JA et al.,2003). Gaede et al reported that vitamin E (680 mg/day) and C (1250 mg/day) combination significantly improved renal function in Type 2 diabetes (Gaede P et al., 2001).

Oxidative stress neurotoxicity

Diabetes-associated oxidative stress is clearly manifest in peripheral nerve, dorsal root, and sympathetic ganglia of the peripheral nervous system and endothelial cells and is implicated in nerve blood flow and conduction deficits, impaired neurotrophic support, changes in signal transduction and metabolism, and morphological abnormalities characteristic of peripheral diabetic neuropathy (diabetic peripheral neuropathy). Hyperglycemia has a key role in oxidative stress in diabetic nerve, whereas the contribution of other factors, such as endoneurial hypoxia, transition metal imbalance, and hyperlipidemia, has not been rigorously proven. It has been suggested that oxidative stress, particularly mitochondrial superoxide production, is responsible for sorbitol pathway hyperactivity, nonenzymatic glycation/glycooxidation, and activation of protein kinase C. However, this concept is

not supported by in vivo studies demonstrating the lack of any inhibition of the sorbitol pathway activity in peripheral nerve, retina, and lens by antioxidants, including potent superoxide scavengers.

Oxidative stress nephrotoxicity

Oxidative stress and related LPO were believed to be involved in the pathogenesis and progression of lupus nephritis, mesangial proliferative glomerulonephritis and hyperlipidemia-induced renal damage in animal models (Yahya MD et al., 1996; Budisavljevic MN et al., 2003; Scheuer H et al., 2000). Clinical studies also revealed the role of LPO in chronic human nephritis. There was a correlation between the activity of various chronic nephropathies and LPO severity in the red blood cells of these patients (Turi S et al., 1997). In diabetic nephropathy, an increase in the production of free radicals and compromised antioxidant activity were found (Bhatia S et al., 2003). Urinary and plasma malondialdehyde (MDA), one of the LPO end-products, were elevated in patients with primary and diabetic nephropathy (Agarwal R.2003). More recently, Fornasieri et al demonstrated the presence of circulating antibodies against oxidatively-modified low density lipoprotein (LDL) in patients with IgA nephropathy (IgAN), focal glomerulosclerosis and primary membranous glomerulonephritis (Fornasieri A et al 2002). In human renal biopsies, oxidized-LDL was shown to be mainly present in the glomerulosclerosis lesions and mesangial areas and it had an obvious clinical significance (Lee HS et al 1998). In diabetic nephropathy, the concentration of oxidized-LDL immune complexes increases and is associated with proteinuria (Atchley DH et al., 2002). Taken together, it demonstrated the presence of oxidative stress in human nephrosis and its involvement in the mechanism of immune injury and clinical manifestation.

Excessive reactive oxygen metabolites can play an important role in the pathogenesis of various renal diseases (Malle E et al 2003; Ha H et al., 2003; Gaertner SA et al., 2002).

Reactive oxygen species could be generated from infiltrating macrophages, resident mesangial and activated neutrophils (Andreoli SP, 1995; Nakamura K et al., 1998. They can attack macromolecules including protein, DNA and lipids etc, leading to cellular/tissue damage.

Fructose Hyperlipidemia

There is considerable evidence supporting the ability of high fructose diets to up regulate the lipogenesis pathway, leading to increased TG production (Kok N et al., 1996). Insulin and glucose are known to directly regulate lipid synthesis and secretion. Insulin controls hepatic sterol regulatory element binding protein (SREBP) expression, which is a key transcription factor responsible for regulating fatty acid and cholesterol biosynthesis. SREBP binds to sterol responsive elements (SRE) found on multiple genes, and can activate a cascade of enzymes involved in cholesterol biosynthetic pathways, such as HMG-CoA reductase (Brown MS and Goldstein JL 1997) and fatty acid synthase (FAS) (Bennett MK et al., 1995). Expression of SREBP is enhanced by insulin in all three major insulin target tissues, liver, fat, and skeletal muscle (Sewter C et al 2002, Kim JB et al 1998, Guillet-Deniau I et al 2005). Similarly, levels of SREBP are enhanced in the presence of hyperinsulinemia (Boizard M et al 1998, Shimomura I et al., 1999). Glucose feeding causes a short-term peak induction, whereas fructose caused a gradual extended increase in SREBP-1c activity, providing evidence that lipogenesis can be independent of insulin signaling, given carbohydrate, and particularly fructose, availability(Matsuzaka T et al 2004). Emerging evidence suggests that a protein phosphatase, known as PTP-1B, may link high carbohydrate feeding, insulin resistance, and lipogenesis. PTP-1B may regulate the lipogenesis and hypertrigylceridemia associated with insulin resistance syndrome (Shimizu S et al 2003). In insulin resistant fructose fed rats, it has been reported that the increase of hepatic SREBP-1 mRNA (Nagai Y et al., 2002) occurs in correlation with an increased PTP-1B expression ((Shimizu S et al 2003). Studies using animal models of insulin resistance, for example, the Wistar fatty rats, showed the effects of dietary carbohydrates on TG production. Feeding fructose to rat stimulated FAS, and created a 56% increase in TG secretion rate, and an 86% increase in plasma TG. Feeding glucose, however, did not have this effect on TG production, nor did it affect induction of FAS. This is likely because glucose stimulates both TG production, and TG removal, maintaining homeostasis. Fructose stimulates TG production, but impairs removal, creating the known dyslipidemic profile (Kazumi T et al 1997).The human liver possesses a large capacity to metabolize fructose to lipids because of its ability to shunt metabolism toward serum TG production. TG stores supply an energy 'sink', providing an almost unlimited TG production capacity. Conversely, glucose as opposed to fructose would decrease serum TG (Herman RH et al 1970). Hirsch

argued that carbohydrate overload results in elevated TG because the large amounts of sugar that need to be absorbed so rapidly from the intestine lead to the involvement of other metabolic pathways, such as the hexose monophosphate shunt, that favor the synthesis of FFA (Hirsch 1995). Again, the liver takes up dietary fructose rapidly where it can be converted to glycerol-3-phosphate. This substrate favours esterification of unbound FFA to form the TG (Fried SK and Rao SP 2003). It has also been found that increases of 1, 2-sn-diacylglycerol and elevated expression of a PKC isoenzyme are associated with the enhanced synthesis of TG observed with high fructose diet models (Donnelly R et al 1994).In these scenarios, where there is excess hepatic fatty acid uptake, synthesis and secretion, 'input' of fats in the liver exceed 'outputs', and hepatic steatosis occurs (Koteish, and Diehl AM 2001). All of these factors contribute to fructose being a highly lipogenic nutrient. There is growing evidence that the insulin resistant state developed upon fructose feeding is also associated with stimulated hepatic VLDL secretion. Excess VLDL secretion has been shown to deliver increased fatty acids and TG to muscle and other tissues, further inducing insulin resistance (Zammit VA et al 2001). The metabolic effects of fructose occur through rapid utilization in the liver due to the bypassing of the regulatory phosphofructokinase step in glycolysis. This in turn causes activation of pyruvate dehydrogenase, and subsequent modifications favoring esterification of fatty acids, again leading to increased VLDL secretion (Mayes PA 1993). Increases in VLDL secretion can then lead to chain reactions in other lipoproteins and lipids, such as low density lipoprotein (LDL). Comparison of plasma lipoproteins from fructose-fed animals showed a significant shift toward secretion of larger, less dense, chylomicrons in the insulin resistant animals.

Free fatty acids

Free fatty acid (FFA) elevation is known to be associated with IR, MS, PD, and T2DM. The metabolically active form of FFAs are cytosolic long-chain acyl-CoA esters (LCACoA) and are responsible for cytosolic neutral triglyceride deposition in adipose and non-adipose tissues.

In 2001, McGarry gave an excellent presentation at the American Diabetes Association meeting (ADA 2001 Banting Lecture), discussing in detail how toxic FFA and LCACoA may be important in the development of insulin resistance,

progressive beta cell dysfunction and death associated with T2DM(McGarry JD,2002).

Central obesity is associated with increased cytosolic neutral fat triglyceride stores in adipose and non-adipose tissues such as muscle (skeletal and cardiac), the liver, pancreatic beta cells and, possibly, endothelial cells (Bakker SJ et al., 2000; McGarry JD, 2002).

Intra-myocellular lipid was found to be more highly correlated with insulin resistance than any other commonly measured indices such as body mass index, waist-to-hip ratio or total body fat. Low insulin sensitivity was accompanied by a marked increase in intra-myocellular lipid. Bakker *et al.* (2000) proposed that the chronic low-grade production of ROS produced by respiring mitochondria is enhanced by excessive cytosolic triglyceride stores and LCACoA esters in non-adipose tissue.They proposed that LCACoA esters exert an inhibitory effect on the adenosine nucleotide translocator with a resultant decrease in the ADP available. This decrease in ADP slows the flow of electrons along the electron transfer chain and increases the possibility of having single unpaired electrons to create the superoxide anion (O_2^-) increasing oxidative mitochondrial stress, thus resulting in a dysfunctional cell. Moreover, they suggest that these phenomena not only accelerate the atherosclerotic process but also induce endothelial dysfunction and microalbuminuria prior to the development of T2DM and possibly beta cell dysfunction and failure.

It is difficult to completely separate FFA toxicity from the sections which follow on lipoprotein toxicity and triglyceride toxicity as there is a dynamic relationship between these three in the A-FLIGHT toxicities. In fact, FFAs are transported by the protein fraction, albumin, and lipases are constantly removing the long chain fatty acids from the glycerol backbone of triglycerides at the interface of the capillary endothelial cells creating free fatty acids which can freely move into cells throughout the body. Intracellularly, the FFAs are then added to the glycerol backbone in order to form cytosolic triglycerides stored as neutral fat, or are oxidized for fuel and energy generating ATP. If mitochondrial beta oxidation is over utilized or dysfunctional, the excess may then undergo the toxic non-beta non-mitochondrial pathway generating toxic FFAs or ceramide.

Lipotoxicity – *generalized*: lipotoxicity promotes oxidative stress and is associated with MS, IR, PD, and T2DM. There is an associated defect of lipoprotein metabolism frequently referred to as the "lipid triad". Elevated VLDL or triglycerides, atherogenic small dense LDL, and decreased HDL comprise this triad which is associated with atherosclerosis and coronary heart disease as well as increased redox stress (Gotto AM Jr.1998; Grundy SM, 1998).

The increased VLDL, triglycerides, atherogenic small dense LDL cholesterol and the diminished amount of the anti-atherogenic, antioxidant anti-inflammatory high density lipoprotein cholesterol would reduce the natural antioxidant reserve. This combination supports an increase in redox stress in addition to the previously discussed FFA toxicity. This also tends to support the oxidation, glycation and glycoxidation of existing lipoproteins (modification) which results in increased ROS and redox stress.

Lipoproteins have the function of transporting lipids throughout the body. Low density lipoproteins are responsible primarily for the transport of cholesterol with the protein moiety involved: apolipoprotein (Apo) B 100. Very low density lipoproteins are responsible for the transport of triglycerides with the protein moiety involved: Apo E. High density lipoproteins are responsible for reverse cholesterol transport and play an important role in being a naturally occurring potent anti-inflammatory and antioxidant agent with the protein moiety involved: Apo A. It is the protein moiety of the lipoproteins that is modified by the processes of oxidation, glycation, and glycoxidation with a resultant increase in redox stress and the production of ROS. Furthermore, the modification of the protein moiety is responsible for their retention within the intima, inducing atherogenesis and thus atherosclerosis. (Williams KJ and Tabas I 1995; 1998).

Lipotoxicity – specific: Lipotoxicity is also associated with MS, IR, PD, and T2DM. Unger *et al* (2001) feel this specific lipotoxicity is attributed to products of the excessive non-beta- (non-mitochondrial) oxidative metabolism of FFA in the skeletal and the myocardial muscle, the liver and the pancreatic islets (Unger RH and Orci L.2001; Lee Y et al 1994;1997; Shimabukuro M et al 1998).In addition, these toxic metabolic products are thought to cause the complications of MS, IR, PD, and T2DM by creating cellular dysfunction and, in time, promoting programmed cellular death

(lipoapoptosis) (Shimabukuro M et al., 1998; 1998). In the normal state, FFA delivery to non-adipose tissue is closely regulated to its need for fuel. FFAs normally rise during exercise and fasting in order to meet metabolic requirements and thus, homeostasis is maintained. However, as a result of over-nutrition (western diet), the FFA influx may exceed FFA usage and FFA non-beta oxidation ensues. These non-mitochondrial FFA metabolites, which are responsible for injuring cells, result in lipoapoptosis, include triglycerides, ceramide, and products of lipid peroxidation. Ceramide (an amino alcohol with a LCACoA attached to the amino group) has been implicated for some time in the apoptotic pathway of the T1DM autoimmune destruction of beta cells by sphingomyelin degradation (Obeid LM et al., 1993).

Triglyceride toxicity: Multiple lipases (intestinal, muscular – skeletal and cardiac-, adipose, and hepatic) are responsible for the dynamic flux between the long chain fatty acids (LCACoA esters) and the glycerol molecular backbone of the triglycerides.

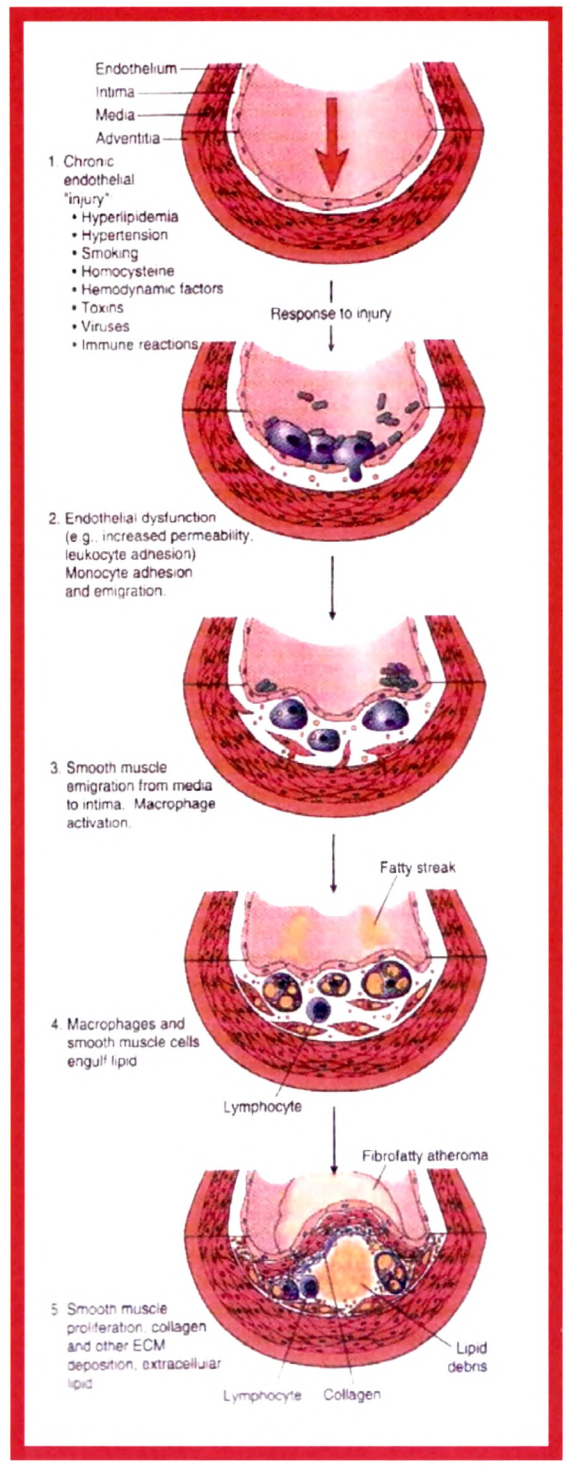
Hypertriglyceridemia certainly plays a role in toxicity regarding the development of redox stress, not only its role in lipotoxicity and FFA toxicity discussed previously, but independently as its own marker of toxicity. There is a close association of hypertriglyceridemia and the atherogenic small dense LDL cholesterol particles which are more likely to be oxidized and contribute to redox stress. This condition is central to the lipid triad (Kahn SE et al 1999).We need to recall that Apo E is responsible for carrying this lipid fraction and that the Apo E knockout mouse develops atherosclerosis at an accelerated rate. We need to also bear in mind that gene polymorphism may play a role in the development of ADIA since Apo E is an important part of all amyloid formation and stabilization. Kahn *et al.* were able to demonstrate in the human islet amyloid polypeptide transgenic mouse model that these mice did not develop islet amyloid unless fed a high fat diet (Wang F et al 2001).Stored neutral triglycerides provide the substrate for FFA production which can be immobilized immediately by exercise or stress induced lipolysis.

Atherosclerosis:

Atherosclerosis is a complex, multifactorial disease in which the cellular and molecular mechanisms contributing to the disease process are poorly defined. Recent insights into the pathogenesis of atherosclerosis suggest that it may be viewed as a

chronic inflammatory disease with an underlying abnormality in redox-mediated signals in the vasculature (Medford RM 1995; Offermann MK and Medford RM 1994; Ross R.1995). In a normal, healthy state, the vessel wall is composed of a single-cell–thick EC lining that exhibits intimate contact with the medial layer of vascular SMCs (VSMCs). Encircling this is the adventitial layer consisting of a dense matrix of connective tissue. As such, the EC is optimally situated at the interface between the circulating blood and the vessel wall to serve as a sensor and transducer of signals within the circulatory microenvironment. Therefore, the EC is integral in maintaining the homeostatic balance of the vessel through the production of factors that regulate vessel tone, coagulation state, cellular proliferative response and leukocyte trafficking. In vascular disease, however, EC dysfunction occurs when the cell loses its ability to maintain the normal homeostatic balance, ultimately leading to impairment in vasorelaxation and increased adhesiveness of the EC lining for circulating inflammatory cells (Ross R.1995).

Oxidative stress and endothelial toxicity: There are a variety of intracellular sources for free radicals and ROS that have been identified. These include, but are not limited to, normal products of mitochondrial respiration, NADPH oxidase, nitric oxide (NO) synthases, cyclooxygenases, lipoxygenases, cytochrome P-450 monooxygenase, and xanthine oxidase (Freeman BA and Crapo JD 1982; Kehrer JP 1993).Via the action of these enzymatic sources and the autoxidation of various soluble cellular biomolecules, eukaryotic cells continuously produce ROS, including superoxide anion (O_2z_2), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OHz). The literature supporting the involvement of various sources of ROS and the nature of individual reactive species has been generalized from many different in vitro and in vivo systems. NADPH oxidase-like activity appears to be a contributing source of ROS via the generation of superoxide anion in both cultured ECs(Mohazzab KM 1994; Bayraktutan U 1998) and VSMCs, (Griendling KK 1994)as well as in intact aortas (Pagano PJ 1995). Cytokines and growth factors such as tumor necrosis factor-a (TNF-a), interleukin (IL)–1b, angiotensin II (Ang II), and interferon-g activate membrane-bound NADPH oxidase to produce superoxide in ECs (De Keulenaer GW1998).


Endothelium
Intima
Media
Adventitia
1. Chronic endothelial "injury"
• Hyperlipidemia
• Hypertension
• Smoking
• Homocysteine
• Hemodynamic factors
• Toxins
• Viruses
• Immune reactions
Response to injury
2. Endothelial dysfunction (e.g., increased permeability, leukocyte adhesion) Monocyte adhesion and emigration.
3. Smooth muscle emigration from media to intima. Macrophage activation.
Fatty streak
4. Macrophages and smooth muscle cells engulf lipid
Lymphocyte
Fibrofatty atheroma
5. Smooth muscle proliferation, collagen and other ECM deposition, extracellular lipid
Lipid debris
Lymphocyte
Collagen

Hyperlipidemia-induced Oxidative Stress and Atherosclerosis

It has long been identified that hyperlipidemia is a major inducer of oxidative stress and plays a pivotal role in atherogenesis in susceptible animals and humans(Hennig B et al.,2001; Mertens A et al., 2003; Yamaguchi Y et al., 2002).In hyperlipidemic patients, the enrichment of triglycerides and the high apo-B cholesterol levels suggest the presence of abnormally high levels of LDL particles(Schreier LE et al 1996).Also, elevated levels of ox-LDL3 in plasma of patients with atherosclerosis further suggest the importance of ox-LDL in hyperlipidemic patients. Increasing evidence has shown that ox-LDL is not only a marker of oxidative stress, but itself can induce oxidative stress in vascular tissues (Mehta JL et al., 2003; Meilhac O et al., 2000). A large body of literature has confirmed a central pro-atherogenic role of ox-LDL in vascular cells (Li Det al ., 2002, 2003; Rosenson RS et al 2002; Ananyeva NM et al 1997). It has been shown that ox-LDL induces pro-atherosclerotic NADPH oxidase expression and superoxide anion formation in human vascular endothelial cells(Rueckschloss U et al 2002),and this may be one mechanism by which ox-LDL stimulates ROS generation and the resultant endothelial dysfunction as well as atherosclerosis.

Since hyperlipidemia is a major risk factor for atherosclerosis, therapies directed at modulating hyperlipidemia may have anti-atherogenic effect. Both probucol and 3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are effective lipid lowering agents. Clinical studies in humans show that these agents can significantly reduce the plasma LDL cholesterol level. The LDL cholesterol-lowering effect may be responsible for their anti-atherosclerotic effects. However, a large number of studies have demonstrated that their anti-atherosclerotic effects are due not only to their lipid-lowering property, but also to their direct anti-oxidant effect (Doggrell SA 2001; Li D et al 2003). HMG-CoA reductase inhibitors in particular provide endothelial stabilization through mechanisms that go beyond their primary lipid-lowering effect. The improvement of endothelial dysfunction induced by ox-LDL may be secondary to a reduction in the production of free radicals that might result in an increase in endothelial NO synthase (eNOS) expression and activity. In addition, HMG-CoA reductase inhibitors have been shown to reduce the susceptibility of LDL to oxidation (Bellosta S et al 2000). These data provide indirect

evidence supporting an anti-oxidant effect of these agents, independent of their lipid-lowering effect.

The effects of ox-LDL on vascular tissues are mediated by specific receptors on monocytes/macrophages and SMCs (Zhou YF et al 1996).Uptake of ox-LDL through these receptors leads to foam cell formation, SMC proliferation and migration and neointima formation. The inhibition of these scavenger receptors indeed is associated with a reduction in atherosclerosis (Jalkanen J et al 2003).

HMG-CoA reductase inhibitors such as simvastatin and atorvastatin inhibit the endothelial uptake of ox-LDL and reduce the upregulation of endothelial ox-LDL receptor LOX-1, and upregulate the expression of eNOS (Mehta JL et al 2001; Li D et al 2001). All these effects may complement the inhibitory effect of statins on ROS generation.

Insulin Resistance and Hypertension

INSULIN (Ins) exerts important biological effects on cardiovascular tissue as well as conventional Ins tissues such as skeletal muscle and adipose tissue (Gupta S et al 1994; 1996; Hansson L et al 1999). For example, Ins and its homologous autocrine/paracrine peptide Ins-like growth factor (IGF-1) induce vasorelaxation by mechanisms that include stimulation of nitric oxide (NO) production and reductions in vascular smooth muscle cell (VSMC) intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and Ca^{2+}-myosin light chain (MLC) sensitization(Isenovic E et al 2002; Isenovic ER et al 2003; Montagnani M et al 2001). IGF-1, unlike Ins, is produced by VSMC and cardiomyocytes in response to angiotensin II (ANG II) and other growth factors and mechanical forces such as stretch (Walsh MF et al., 1996; Standley P et al., 1997). Many of the metabolic and vasomotor effects of Ins and IGF-1 are mediated by activation of the phosphatidylinositol 3-kinase (PI3K) and downstream signaling pathways, including protein kinase B (Akt) (Zeng G et al., 2000; Kim YBet al ., 1999). The serine-threonine kinase Akt interacts with the phospholipids produced by PI3K, thereby undergoing phosphorylation of Thr^{308} and Ser^{473}, which results in its activation. The activation of Akt is a necessary but not definitive requirement for Ins and IGF-1 to exert their metabolic and vascular effects. Vascular relaxation in response to activation of PI3K/Akt signaling is mediated in part by endothelial cell production of NO (Walsh MF et al., 1996; Zeng G et al ., 1996). Another effect of

Ins/IGF-1 stimulation of the PI3K/Akt signaling pathway is a reduction in VSMC $[Ca^{2+}]_i$ and Ca^{2+}-MLC sensitization(Sandu OA et al .,2001; Standley PR et al 1991). Ins and IGF-1 reduce VSMC $[Ca^{2+}]_i$ by inhibiting agonist-induced inward Ca^{2+} currents and intracellular organelle release of Ca^{2+} (Ouchi Y et al., 1996; Standley PR et al.,1993). Ins and IGF-1 also reduce $[Ca^{2+}]_i$ by stimulating the activity of the Na^+-K^+-ATPase pump in VSMC, a process that is dependent on PI3K/Akt signaling(Hansen LL et al.,1999; Li D et al., 1999; Tirupattur PR et al.,1993). Thus Ins and IGF-1 induce vascular relaxation by stimulation of endothelial cell production of NO and by reducing VSMC $[Ca^{2+}]_i$ and Ca^{2+}-MLC sensitization. These effects are mediated, in part, by activation of PI3K/Akt signaling pathways. This signaling pathway is also necessary for Ins and IGF-1 stimulation of glucose transport in vascular skeletal muscle and adipose tissue (Kim YB et al., 1999; Standley PR et al 1995).

Hypertention and oxidative stress

Angiotensin II increases vascular oxidative stress as well as vasoconstriction (Higashi Y et al., 2002; Dzau VJ et al., 2001; Mollnau H et al 2002). Activation of the renin–angiotensin system in tissue(Lerman LOet al 2001), as well as increase in the systemic renin–angiotensin system, enhances the vascular production of reactive oxygen species, in part through the activation of membrane-bound NADH and NADPH oxidases (Rajagopalan S et al 1996; Mollnau H et al 2002).These oxidase enzymes are present in endothelial cells, vascular smooth-muscle cells, fibroblasts, and phagocytic mononuclear cells.The increased vascular activity of NADH and NADPH oxidase enhances the production of reactive oxygen species by several pathways, including the increased activation of xanthine oxidase, the auto-oxidation of NADH, and the inactivation of superoxide dismutase. There is increasing evidence that the resultant increase in nitric oxide degradation or inactivation by reactive oxygen species, rather than reduced nitric oxide production itself, plays the principal part in the impairment of endothelium-dependent vasodilatation in diabetes(Sowers JR et al., 2001)and other vascular diseases(McIntyre M et al 1999; Berry C et al 2000; Irani K 2000) characterized by enhanced tissue activation of the renin–angiotensin system(Berry C et al 2000; Irani K 2000).Thus, enhanced production of reactive oxygen species (including oxygen radicals) causes a loss of bioavailability of nitric oxide, which impairs endothelium-dependent vasodilatation. The reaction of oxygen

radicals with nitric oxide leads to the production of peroxynitrite (Irani K 2000),a potent oxidant that further contributes to vasoconstriction and vascular injury(Dijkhorst-Oei LT et al 1999).Although the reduction in markers of oxidative stress and the associated improvement in endothelium-dependent vasodilatation after angioplasty may be due to reductions in tissue and circulating angiotensin II, the reduction in blood pressure after angioplasty may also have an important role(Cai H et al., 2000; Hornig B et al., 2001; Zalba G et al.,2001).Studies in animals with hypertension and in humans with essential hypertension have shown that endothelial dysfunction is associated with an excess of oxygen radicals and increased production of reactive oxygen species (Berry C et al 2000; Irani K 2000). It has been suggested (McIntyre M et al 1999) that in essential hypertension there is an imbalance between enhanced production of oxygen radicals and decreased antioxidant activity. Indeed, the levels of free-radical scavengers, such as glutathione, superoxide dismutase, and vitamin E, have been reported to be depressed in patients with hypertension (Irani K 2000).

Role of hypertension in target organ damage (Ronald G. al., 2005):

A. Cardiac

1. Left ventricular hypertrophy
2. Systolic and diastolic dysfunction
3. Congestive heart failure
4. Coronary artery disease

B. Cerebrovascular

1. Strokes (acute and chronic stroke)
2. Carotid stenosis
3. Dementia

C. Renal disease

1. Acute renal disease
2. Chronic renal disease, diabetic and nondiabetic
3. Progression of renal damage

D. Other vascular diseases

1. Atherosclerotic: aneurysms, dissection embolization
2. Fibromuscular dysplasia
3. Vasospastic and inflammatory
4. Peripheral arterial disease

E. Others

1. Retinopathy
2. Sexual dysfunction

Myocardial infarction

Ischemic heart disease is caused by an imbalance between the myocardial blood flow and the metabolic demand of the myocardium. Reduction in coronary blood flow is related to progressive atherosclerosis with increasing occlusion of coronary arteries. Blood flow can be further decreased by superimposed events such as vasospasm, thrombosis, or circulatory changes leading to hypoperfusion.

The pathogenesis can include:

- Occlusive intracoronary thrombus - a thrombus overlying an ulcerated or fissured stenotic plaque causes 90% of transmural acute myocardial infarctions.
- Vasospasm - with or without coronary atherosclerosis and possible association with platelet aggregation.
- Emboli - from left sided mural thrombosis, vegetative endocarditis, or paradoxic emboli from the right side of heart through a patent foramen ovale.

The gross morphologic appearance of a myocardial infarction can vary. Patterns include:

- Transmural infarct - involving the entire thickness of the left ventricular wall from endocardium to epicardium, usually the anterior free wall and posterior free wall and septum with extension into the RV wall in 15-30%. Isolated infarcts of RV and right atrium are extremely rare.

- Subendocardial infarct - multifocal areas of necrosis confined to the inner 1/3-1/2 of the left ventricular wall. These do not show the same evolution of changes seen in a transmural MI.

Animals develop 'infarct-like' lesions when injected with isoproterenol (ISO), a potent synthetic catecholamine. These lesions are morphologically similar to those of 'coagulative myocytolysis' (COAM) or myofibrillar degeneration, one of the findings in acute myocardial infarction in man (Milei J et al 1973). Diabetic patients are more vulnerable to myocardial damage resulting in heart failure than nondiabetic patients (Richard W et al., 1998).It is suggested that heart failure subsequent to myocardial infarction may be associated with an antioxidant deficit, as well as increased myocardial oxidative stress (Hill MF and Singal PK 1996). Higher serum enzyme concentrations of aspartate transaminase (AST) and creatinine kinase (CK) act as markers and are associated with higher incidence of stroke after acute myocardial infarction(Thompson PL and Robinson JS 1978). Elevated serum uric acid lactate dehydrogenase (LDH), creatinine kinase-MB fraction (CK-MB) may also act as marker enzymes of tissue ischemia (Waring WS et al .,2000). An Increase in concentration of total cholesterol and LDL cholesterol and a decrease in HDL cholesterol are associated with raised risk of myocardial infarction (Mediene-Benchekoret at al., 2001).

α_1-Adrenoceptor:

Since their original classification of adrenergic receptors into stimulatory and inhibitory receptors and subdivision into α_1- and α_2-ARs, it became apparent that there was heterogeneity in α_1-ARs. Indeed, prior to the cloning of any receptor subtypes, numerous reports provided functional evidence of α_1-AR heterogeneity. McGrath was the first to suggest subdividing the α_1-ARs into α_{1A}- and α_{1B}-ARs. Morrow and Creese noted that the inhibition curves for a series of agonists and antagonists to displace [3H] prazosin were biphasic. Since these initial studies, the α_{1A}-subtype was pharmacologically classified to have higher binding affinity for agonists, such as methoxamine and oxymetazoline, and antagonists, such as 5-methylurapidil, niguldipine, and WB4101. In contrast, the α_{1B}-AR subtype had lower binding affinity for the above ligands (Paul A et al., 1996, Minneman et al., 1988).

After these initial pharmacological studies, the first cDNA cloned was the hamster α_{1B}-AR. This cDNA had all of the pharmacological properties of the tissue-

characterized α_{1B}-AR and has never been questioned in its classification. The next receptor cloned was called the α_{1C}-AR and was thought to represent a novel subtype. However, it was later reclassified to be the tissue-type α_{1A}-AR. The confusion was centered on its inability to localize its mRNA to tissues known to express the α_{1A}-AR. The next cDNA cloned was initially termed the α_{1A}-AR but also later was reclassified to a novel subtype called the α_{1D}-AR. In this case, the confusion was due to an incomplete pharmacological profile. With the discovery of the α_{1D}-AR, its binding and functional properties were compared with the previously known tissue subtypes. The α_{1D}-AR has a binding profile much like the α_{1B}-AR. Recently an α_{1D}-AR-selective drug has become available.

Characteristics of α_1-Adrenorecetor (Paul A et al., 1996):

A summary of current characteristics of α_1-Adrenorecetor subtypes is shown in the table below.

Characteristics of α_1-AR subtypes

	α_{1A}	α_{1B}	α_{1D}
Previously called	α_{1C}	–	α_{1AD}, α_{1A}
Species cloned	Human, rat, mouse, rabbit, dog, pig, guinea pig, bovine	Human, rat, mouse, guinea pig, hamster	Human, rat, mouse, pig, rabbit
Binding			
Selective antagonists (>50×)	(+)Niguldipine, 5-methylurapidil KMD-3213	–	BMY 7378
Signaling			
PLC, PLA_2, PLD, Ca^{2+}, K channels, MAPKs	Coupling efficiency ++++	++	+
Desensitized internalized	++	+++	±
Protein (amino acids)	465 (rat), 466 (human)	515 (rat), 519 (human)	560 (rat), 572 (human)
Characteristic tissues (rat)	Vasculature, vas deferens, urethra, submaxillary gland	Liver, spleen	Aorta, iliac, femoral arteries
Cellular localization	Cell surface and intracellular	Cell surface	Mostly intracellular

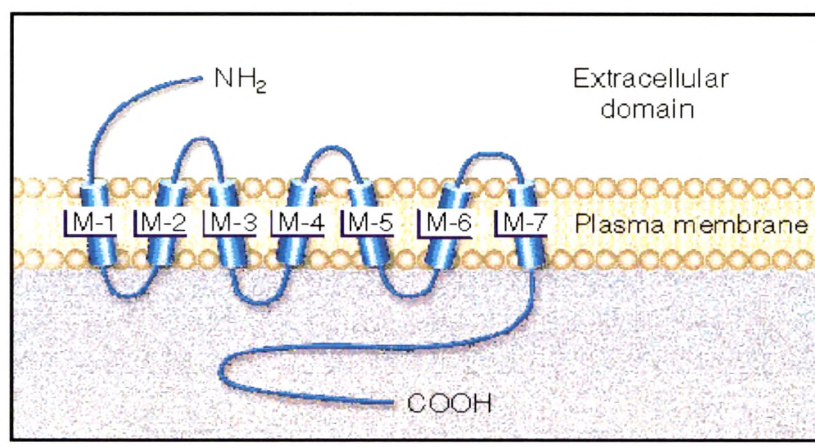
Pharmacological ligand binding profile of various expressed alpha l AR subtypes (Jon W et al., 1991)

	Rat α_{1A}	Rat α_{1B}	Bovine α_{1C}
Agonists			
(−)Epinephrine	546	4,690	6,250
(−)Norepinephrine	100	10,500	9,730
(+)Epinephrine	18,100	ND	ND
Methoxamine	110,000	1,610,000	203,000
Phenylephrine	1,440	23,900	47,800
Oxymetazoline	2,140	824	114
Antagonists			
Prazosin	0.33	0.56	0.37
Phentolamine	111	340	15.3
Indoramine	611	226	ND
Corynanthine	253	517	142
WB4101	2.1	28.6	0.68

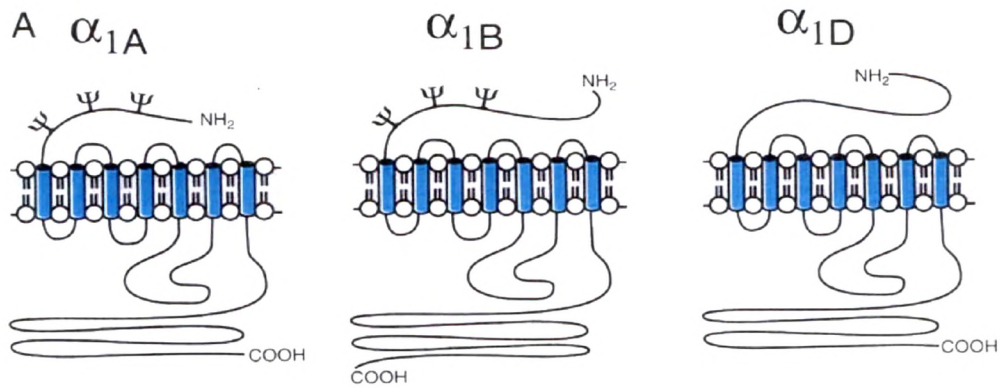
Structure of Adrenergic Receptor; (Paul et al., 1996)

The adrenergic receptors constitute a family of closely related proteins. They also are related both structurally and functionally to receptors for a wide variety of other hormones and neurotransmitters that are coupled to G proteins.

Proposed Arrangement of an Adrenergic Receptor the Plasma Membrane.



Subtypes of α_1-adrenergic receptor;



This wider family of receptors includes receptors not only for catecholamines and other small molecules (such as acetylcholine, dopamine, histamine, and prostaglandins) but also for peptides (such as vasopressin, oxytocin, and angiotensin), proteins (such as glucagon, follicle-stimulating hormone, luteinizing hormone, and thyrotropin), odorants, light, and taste molecules. Thus, adrenergic receptors probably evolved from a common ancestor along with the receptors that recognize other hormones and neurotransmitters or environmental stimuli. All G-protein–coupled receptors share the following structural features: extracellular amino terminals with sites for N-linked glycosylation, seven α-helical domains that are each thought to span the plasma membrane, and intracellular carboxy terminals containing amino acid sequences that indicate probable sites of phosphorylation by one or more protein kinases. M-1 through M-7 denotes the seven α-helical membrane-spanning regions that create three intracellular and three extracellular loop domains. This arrangement is proposed for adrenergic receptors and other types of G-protein–linked receptors that span plasma membranes. Like other member of this gene family, α_1ARS are single polypeptide chains, ranging from 429 to 561 amino acids in length. There is no evidence that the polypeptide chain is posttranslationally processed, although it is posttranslationally modified by the attachment of oligosaccharides (and probably fatty acids also) as well as phosphorylated. There is no evidence for clearly defined leader sequence, so membrane insertion most likely involves the use of cryptic signal sequences.

Each receptor contains seven stretches of 20 to 28 hydrophobic amino acids that likely represent membrane-spanning regions. In several instances, these

hydrophobic stretches are interrupted by charged residues that are functionally important for ligand binding and signaling. The amino termini of α_1ARS are located extracellularly and contain several consensus sites for modification by N-linked glycosylation. The amino termini vary considerably in length, with the terminus for the α_{1D}AR being much longer (90 amino acids) than the terminus for the $\alpha_{1A/c}$AR (25 amino acids) or the α_{1B} AR (42 amino acids). This longer amino terminus of the α_{1D} AR may limit efficient translation or membrane insertion, since this subtype is more poorly expressed in the plasma membrane than the other two subtypes.The carboxy termini are located intracellularly and contain consensus sites for phosphorylation by serine/threonine protein kinases, and modification of the proteins at these sites is involved in receptor desensitization. The carboxy terminal regions show little homology among the subtypes.

The transmembrane-spanning regions are linked by three intracellular and three extracellular loops. These loops, although variable, are each very similar in length among the subtypes. The first and second extracellular loops each contain a single cysteine residue, and analogous cysteines are highly conserved in all GPCRs. In the β-AR and in rhodopsin, these cysteine residues are essential for the correct folding of the proteins, for maturational glycosylation, and for expression in the plasma membrane. This is due to the involvement of these cysteines in a disulfide bond(s). The α_{1B} AR also has been shown to contain an essential disulfide bond, which is solvent inaccessible. Like other member of the GPCR family, the second and third intracellular loops are likely to be involved in signaling through an interaction with receptor-coupled G proteins.

Saponins

The saponins are naturally occurring surface-active glycosides. They are mainly produced by plants, but also by lower marine animals and some bacteria (Riguera, 1997; Yoshiki et al. 1998). They derive their name from their ability to form stable, soap-like foams in aqueous solutions. This easily observable character has attracted human interest from ancient times. Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked

to a hydrophobic aglycone (sapogenin) which may be triterpenoid or steroid in nature. The aglycone may contain one or more unsaturated C–C bonds. The oligosaccharide chain is normally attached at the C3 position (monodesmosidic), but many saponins have an additional sugar moiety at the C26 or C28 position (bidesmosidic). The great complexity of saponin structure arises from the variability of the aglycone structure, the nature of the side chains and the position of attachment of these moieties on the aglycone. Experiments demonstrating the physiological, immunological and pharmacological properties of saponins have provoked considerable clinical interest in these substances.

The physiological role of saponins in plants is not yet fully understood. Saponins may be considered a part of plants' defense systems, and as such have been included in a large group of protective molecules found in plants named 'phytoanticipins' or 'phytoprotectants' (Morrissey & Osbourn, 1999). There are several strategies available for the isolation of saponins. As a general rule, they begin with the extraction of the plant material with aqueous methanol or ethanol. Further processing of the extract is carried out after evaporation under reduced pressure, dissolution in a small amount of water and phase separation into n-butanol.

Biological effects in animals

Saponins have been known to have a lytic action on erythrocytemembranes and this property has been used for their detection. The hemolytic action of saponins is the result of the affinity of the aglycone moiety for membrane sterols, particularly cholesterol (Glauert et al.1962), with which they form insoluble complexes (Bangham & Horne, 1962). Johnson et al. (1986) found that some saponins increase the permeability of intestinal mucosal cells in vitro, inhibit active mucosal transport and facilitate uptake of substances that are normally not absorbed. Gypsophila saponins in the diet depressed mean liver Fe concentrations and total liver Fe by impairing Fe absorption (Southon et al. 1988). Johnson et al. (1986) found that some saponins increase the permeability of intestinal mucosal cells in vitro, inhibit active mucosal transport and facilitate uptake of substances that are normally not absorbed. Saponins reduce protein digestibility probably by the formation of sparingly digestible saponin–protein complexes (Potter et al. 1993). Endogenous saponins affected the chymotrypsic hydrolysis of soyabean protein, particularly glycinin (Shimoyamada et al. 1998).

Saponins isolated from plants such as fenugreek (Petit et al. 1993), Phellodendron cortex and Aralia cortex (Kim et al.1998c), Pueraria thunbergiana (Lee et al. 2000a), and Calendula officinalis (Yoshikawa et al. 2001) have been shown to have hypoglycaemic effects. Petit et al. (1993) found chronically higher plasma insulin levels, probably caused by stimulation of the β-cells in wistar rats The saponin momordin Ic was also found to significantly and dose-dependently inhibit gastric emptying (Matsuda et al. 1999a).

Saponins also reduced the more harmful LDL-cholesterol selectively in the serum of rats, gerbils and human subjects (Potter et al. 1993; Harris et al. 1997; Matsuura, 2001). Morehouse et al. (1999) found that the mechanism of action of saponins was luminal but did not involve stoichiometric complexation with cholesterol. Ginsenoside Rg3 (20(s) protopanaxadiol type) exhibited a dose-dependent inhibitory activity on the expression of absorption of dietary fat by inhibiting pancreatic lipase activity (Han et al. 2000). Saponins on animal reproduction have long been known and have been ascribed to their abortifacient, antizygotic and anti-implantation properties (Tewary et al. 1973; Stolzenberg & Parkhurst, 1976). Saponins from broom weed (Gutierrezia sp.) and lechuguilla (Agave lecheguilla) or commercial pharmaceuticalgrade saponins caused abortion or death or both in rabbits, goats and cows when administered intravenously at concentrations above 2·3 mg/kg body weight (Dollahite et al. 1962). Saponins isolated from the crude extract of Gleditischia horrida, Costus speciosus Sm and Phytolacca Dodecandra caused sterility in mice (Chou et al. 1971; Tewary et al. 1973; Stolzenberg & Parkhurst, 1976).Quin & Xu (1998) found that the butanol extract of Mussaenda pubescens was capable of terminating pregnancy in rats. Extracts of this plant are used as a contraceptive in the Fujian province of China.However, saponin-rich extracts from Combretodendron africanum injected into female rats stimulated uterine growth, lowered luteinizing hormone release, and blocked the oestrous cycle (Benie et al. 1990). Saponins have been shown to have both positive and negative effects on the viability of human sperm cells in vitro with some ginseng saponins increasing motility as well as progression of sperm (Chen et al. 1998) while Sesbania sesban saponins were spermicidal at 1·0–1·3 mg/ml (Dorsaz et al. 1988). Saponin-based adjuvants have the unique ability to stimulate the cell-mediated immune system, as well as to enhance antibody production, and have the advantage that only a low dose is needed for adjuvant activity (Oda et al. 2000). Saponin-based adjuvants

have the unique ability to stimulate the cell-mediated immune system, as well as to enhance antibody production, and have the advantage that only a low dose is needed for adjuvant activity (Oda et al. 2000). There is evidence that saponins may increase the immune response by increasing the uptake of antigens from the gut and other membranes. Oral administration of Panax ginseng C. A. Meyer saponins (Jie et al. 1984), Quillaja saponins (Maharaj et al. 1986), and the butanol extract of Lonicera japonica (Lee et al. 1998), and de-acylated saponin-1 administered on the nasal mucosa (Recchia et al. 1995), all stimulated the immune responses in vivo Saponins isolated from different plants and animals have been shown to specifically inhibit the growth of cancer cells in vitro (Kuznetzova et al. 1982; Rao & Sung, 1995; Konoshima et al. 1998; Marino et al. 1998; Mimaki et al. 1998a; Podolak et al. 1998). Triterpenoid saponins (avicins from Acacia victoriae) selectively inhibited growth of tumour cell lines by cell cycle arrest in human breast cancer cell line and apoptosis in leukaemia cell line (Mujoo et al. 2001) and reduced both tumour incidence and multiplicity in a murine skin carcinogenesis model (Hanausek et al. 2001). Saponin-initiated cytotoxicity thus begins non-specifically with cell aggregation caused by detergent action (Mimaki et al. 2001), followed by specific toxicity determined by receptors on the cell surface and saponin structure leading to what seems to be a mechanism of apoptosis that is independent of saponin structure. Saponins also reduce occurrence of reactive oxygen species such as H2O2 (Haridas et al. 2001a; Pawar et al. 2001), probably by enhancing its breakdown by activation of peroxiredoxins and catalase, and/or glutathione peroxidase as well as by suppressing its production by inhibiting the hosphatidylinositol- 3-kinase signalling pathway (Haridas et al. 2001a). A group of saponins produced in legumes, namely, group B soyasaponins, contain an antioxidant moiety attached at C23 (Yoshiki et al. 1998). This unique sugar residue, 2, 3-dihydro-2, 5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), allows saponins to scavenge superoxides by forming hydroperoxide intermediates, thus preventing bio-molecular damage by free radicals (Yoshiki & Okubo, 1995; Hu et al. 2002).Ginseng extract has been shown to have neurotrophic and neuroprotective effects (Rudakewich et al. 2001). It significantly improves learning ability and cognitive functions in brain-damaged rats in a dose-dependent manner, and enhances the strategic performance of normal rats. These effects are thought to be due to membrane-stabilising effects such as the inhibition of Na+ and Ca2+ channels (Zhao & McDaniel, 1998). Ginseng total saponins injected intracerebroventricularly inhibit stress-induced hypothalamo–

pituitary–adrenal responses by inducing NO production in the brain (Kim et al. 1998a). Glycyrrhetinic acid, the active aglycone of a liquorice saponin, for example, was found to be a highly efficient inhibitor of corticosteroid 11b-dehydrogenase (Monder et al. 1989) and showed mineralocorticoid-like activity.