Advanced glycation end products (age) for diabetes

Diabetes mellitus is a syndrome which defined as a group of metabolic diseases characterized by hyperglycemia, that result from insufficient production of insulin, or body cells poorly respond to the insulin that is produced, or both. Insulin is a hormone produced in the pancreas and secreted in the blood to maintain blood glucose in the body through enables body cells to absorb glucose, to turn into energy. If the body cells enable to absorb the glucose, the glucose will accumulates in the blood (hyperglycemia), leading to many and different potential medical complications (Harmel & Mathur, 2004).

Diabetes have several categories but the majority of cases fall into two categories which are type 1 diabetes mellitus and type 2 diabetes mellitus. These two types are powerful and highly independent risk factors causing coronary artery disease, stroke, peripheral arterial disease and organ damage and dysfunction including eyes and nerves (Harmel & Mathur, 2004).

Type 1 Diabetes Mellitus:

Type 1 diabetes mellitus resulting from pancreas failure to produce insulin hormone. Person at risk of developing type 1 can be identified by doing serologic test markers that showing evidence of autoimmune destruction of beta cells (islet cells) of the pancreas which is responsible for insulin production. Type 1 diabetes is manifested in childhood and early adulthood, but can patient present at any age (Goroll & Mulley, 2009).

Type 2 diabetes mellitus:

Patients with type 2 diabetes mellitus are prone with wide range of series complications. Type 2 is characterized by high blood glucose due to insulin resistance and relative insulin deficiency. There are 20. 8 million people in United State with type 2 diabetes mellitus. Type 2 diabetes traditionally is seen in elderly people. However it is diagnosed in obese children. Many studies shows that type 2 diabetes mellitus are associated with high calorie diet, physical inactivity and life style (Feinglos & Bethel, 2008).

Other Types of Diabetes:

There are other types of diabetes but they are less common but patients who are underlying defect or disease process can be identified in a relatively specific manner. These types are Genetic defects of beta-cell function, Diseases of the exocrine pancreas e. g Fibrocalculous pancreatopathy, Endocrinopathies and cystic fibrosis and Uncommon forms of immune-mediated diabetes.

Diabetes mellitus complications:

Diabetic complications can be grouped into macrovascular and microvascular disease. Macrovascular diseases are result from atherosclerosis which develops in earlier age in patient with diabetes. There are several factors contribute to atherosclerosis such as lipidemia, hypertension, increased platelets adhesion and aggregation, elevated factor V, factor VII and fibrinogen concentration. Macrovascular diseases are seen in both type one and two of diabetes mellitus and they include coronary

heart disease, Ischemic stroke and peripheral vascular disease (which can lead to ulcers, gangrene and amputation) (Winter & Signorino, 2002).

Whereas, Microvascular complications is seen in type one diabetes mellitus. Hyperglycemia damages the basement membrane of capillaries in the retina and glomerulars which leads to retinopathy and neuropathy. Microvascular diseases include neuropathy (nerve damage), nephropathy (kidney disease) and vision disorders (eg retinopathy, glaucoma, cataract and corneal disease). Furthermore there are other complications of diabetes include infections, metabolic difficulties, dental disease, autonomic neuropathy and pregnancy problems (Winter & Signorino, 2002).

Several clinical research show a strong relationship between hyperglacemia and diabetic microvascular complications in both type 1 and type 2 diabetes. High glucose and insulin resistance play important roles in the pathogenesis of macrovascular complications due to atherosclerosis. Diabetes-specific microvascular disease in the retina, glomerulus and vasa nervorum has same pathophysiological features. Intracellular hyperglycaemia causes abnormalities in blood flow and increased vascular permeability which leads to decreased activity of vasodilators such as nitric oxide, increased activity of vasoconstrictors such as angiotensin II and endothelin-1, and elaboration of permeability factors such as vascular endothelial growth factor (VEGF) (Brownlee, 2001).

There are several factors which contribute the formation of inflammation, atherosclerosis and diabetes mellitus complication. These factors are hyperglycemia, accumulation of advanced glycation endproducts,

dyslipidemia and oxidative stress which lead to endothelial dysfunction resulting in thrombotic complications and cardiovascular (Altman, 2003).

In type 1 diabetes mellitus, high blood glucose is usually as result of low level of insulin secretion whereas in type2 hyperglycemia is caused by resistance of insulin at the cellular level. Low insulin levels or insulin resistance enable the body to convert glucose into glycogen (a starch-as source of energy which stored in the liver).

In type 1 diabetes, pancreatic beta cells are attacked by auto-immune which cause infiltration of inflammatory cells and increased expression and secretion of S100-calcium binding protein and high-mobility group protein 1 (HMGP1) through inflammatory cells which consequently lead to islet damage and increased blood glucose level, subsequent accumulation of AGE (Bierhaus & Nawroth, 2009).

Comparing to T1D, in type 2 diabetes mellitus, different metabolic disturbances stimulates inflammatory cells to secretes RAGE ligands, which cause low-grade inflammation and increased oxidative and carbonly stress, all these play roles in promoting AGE formation and RAGE expression in respective organs (Bierhaus & Nawroth, 2009).

Insulin resistance and Inflammation:

In diabetic patients with hyperglycemia, cause by 1) impaired insulin secretion by the pancreatic ß-cells, 2) muscle insulin resistance, and 3) hepatic insulin resistance all play central roles in the development and progression of glucose intolerance.

As I mention before, type 2 diabetes mellitus (T2DM) is characterized by insulin resistance in liver and muscle and impaired insulin secretion. Also include deranged adipocyte metabolism and modified fat topography in the pathogenesis of glucose intolerance in T2DM. Fat cells are resistant to insulin's antilipolytic effect, which result in increased plasma free fat acid levels. Long period of increasing plasma fat fee acid stimulates gluconeogenesis, promote hepatic and muscle insulin resistance, and impairs insulin secretion in genetically predisposed patients. These pathways of FFA-induced are known as lipotoxicity. Fat cells Dysfunction makes excessive amounts of insulin resistance-inducing, inflammatory, and atherosclerotic-provoking cytokines and fails to secrete normal level of insulin-sensitizing adipocytokines. Enlarged fat cells are insulin resistant and have minimized capacity to store fat. When storage capacity of adipocyte is exceeded, lipid " overflows" into muscle, liver, and perhaps ß-cells, resulting in muscle, hepatic insulin resistance and impaired insulin secretion.

In type 2 diabetics, the ability of insulin to stop lipolysis and to decrease the plasma FFA levels is markedly impaired. It is clearly that chronic elevated levels of plasma free fat acid leads to insulin resistance in muscle and liver, and impair insulin secretion. In addition to FFA in plasma, increase stores of triglycerides in muscle and liver which correlate closely with the presence of insulin resistance in tissues. The triglycerides in liver and muscle are in a state of constant turnover, and the metabolites of intracellular triglyceride lipolysis impair action of insulin in liver and muscle (Bays et al, 2004).

This pathway of events has been referred to as lipotoxicity . The accumulation of lipid in dipocytes leads to activate NADPH oxidase which

increases the production of reactive oxygen species (ROS). The mechanisms increased production of cytokines including TNF-α, IL-6 and monocyte chemoattractant protein-1 and reduce the production of adiponectin (Shoelson et al, 2006).

Furthermore accumulation of lipid activates the protein response to increased ER stess in fat and liver. All these have accumulated to induce lipotoxicity as an important cause of ß-cell dysfunction (Bays et al, 2004).

Receptor for advanced glycation end products (RAGE):

RAGE is type I transmembrane receptor of the immunogloblin superfamily. It is a receptor for advanced glycation endproducts (AGE). It is about 45-KD a protein and it is consist of 403 amino acids in human, rate and mouse. Its extracellular region consists of one V-type (variable) immunoglobulin domina, which followed by two C-type (constant) immunoglobulin dominas (Basta, 2004).

Its name drive from its ability to bind AGE and also it is known as AGER. It is located within the major histocompatibility complex (MHC) class III region on chromosome 6, which contains numbers of genes involved predominantly in inflammatory and immune responses and several components of complements. Beside AGE, RAGE is able to bind several ligands therefore is called a pattern-recognition receptor. These ligands are high-mobility group protein 1 (HMGP1), S100-calicum binding protein (S100b) which is family of pro-inflammatory cytokines and it can be found in any inflammatory lesion and vascular walls of diabetics patients, amyloid-ß-protein and macrophage-1 glycoprotein (CD11B/CD18). RAGE plays role in diabetes and other

metabolic disease. The interaction of RAGE and its ligands cause pro-inflammatory activation. It is involved in several diseases such as innate immune response, mediating immune and inflammatory response, cancer promoting and progressing and microvascular and microvascular diseases (Bierhaus & Nawroth, 2009).

Moreover, RAGE expression is increased in the cells of the vascular walls, at the site where AGEs and S100/calgranulins are accumulated, including endothelium vascular smooth muscle cells, glomerular mesangial cells and mononuclear phagocytes. This destrbution of RAGE and its ligands leads to increased cellular activation, causing further raised expression of the receptor (Basta et al, 2004).

Recently the studies have been proved that RAGE and its ligands accumulate in diabetes and contribute to its pathology. Serum levels of S100A8/9 and S100A12 increased in type 2 diabetic patients. Also, evidence was provided by raised serum levels HMGB1 were linked to coronary artery disease in type 2 diabetes. In addition the studies which have been done in streptozotocin-induced diabetic rats proved that diabetes increased amyloid-beta-peptide (1-40) levels in the brain. Furthermore, CML-modification of S100A8 and S100A9 are seen in inflammatory bowel disease and promote RAGE-mediated sustained inflammatory. Moreover, Carboxylated N-glycans on RAGE eases binding of HMGB and mediates ligation of S100A8/A9 to subpopulation of RAGE on colon cancer cells (Bierhaus & Nawroth, 2009).

Advanced Glycation End products (AGE) and its biochemical mechanism production:

There are various pathways involved in stimulation of atherosclerosis in diabetes mellitus. However the most important one is formation and deposition of AGEs through nonenzymetic reaction between extracellular protein and glucose and it accumulates within cells of the vascular walls, in the extracellular space, kidney, nerves and retina (Basta et al, 2004).

Possible mechanism of AGEs formation arise from intracellular auto-oxidation of glucose to glyoxal, decompounding of the Amadori product (glucose-derived 1-amino 1-deoxyfructose lysine adducts) to 3-deoxyglucosone and fragmentation of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate to methylglyoxal. These reactive intracellular dicarbonyls (glyoxal, methylglyoxal and 3-deoxyglucosone) which can react with amino groups of intracellular and extracellular proteins to form AGEs (pyrraline, pentosidine, CML, crossline) (Balasubramanyam et al, 2002).

Several experimental studies evidenced that advanced glycation end products can alter vascular wall homeostasis in atherogenic through different ways which are:

1. AGEs and mononuclear phagocytes Interaction:

The binding of AGEs with mononuclear phagocytes MPs induces the activation of platelet-derived growth factor, insulin-like growth factor-1, and pro-inflammatory cytokines, such as IL-1ß and TNF-α. Furthermore the interaction of AGEs with its receptor (RAGE) in the mononuclear phagocytes promotes cell migration (chemotaxis) (Basta et al, 2004).

2. AGEs and vascular smooth muscle cells Interaction:

Interaction of AGE with smooth muscle cells (SMCs) exhibits the proliferative activity and production of fibronectin. SMC growth are indirectly mediate by cytokines or growth factors which induced by AGEs in the MPs. Transforming growth factor-ß (TGF-ß) act as an intermediate factor in AGE-induced fibronectin formation by SMC(Basta et al, 2004).

3. AGEs with vascular endothelium Interactions: alterations of vascular permeability and of adhesive properties

As result its unique position and numerous properties, the vascular endothelium has important role in the regulation of extracellular permeability, the maintenance of blood fluidity, metabolism of hormones and vasoactive mediators and the regulation of vascular growth and tone. The endothelium is exposed to AGEs located on circulating proteins and cells (such as, diabetic RBCs), also those found in the underlying subendothelial matrix. Receptors for AGEs present on the endothelial cell surface, and mediate both the uptake and AGEs transcytosis, and the internal signal transduction. AGE-RAGE interaction leads to alteration of barrier function and an increased permeability of endothelial cells interact with AGEs and increased migrate of macromolecules through the endothelial monolayer. The increase in permeability is associated by alterations of the physical integrity of the endothelium, as shown by the destruction of structures and alterations of cellular morphology (Basta et al, 2004).

Also, it has been proved that AGEs cause alterations of endothelial anti-hemostatic functions in vitro, through a reduction of thrombomodulin expression and accompanied with induction of tissue factor expression. The

promoting of tissue factor and the decreasing in thrombomodulin activity change the dynamic endothelial features with regard to hemostasis from those of an anticoagulant to those of a procoagulant surface (Basta et al, 2004).

Intraction of AGEs with endothelial RAGE also causes the depletion of cellular antioxidant defense mechanisms (such as glutathione, vitamin C) and the generation of reactive oxygen species. As a result of the increased cellular oxidative stress, NF-κB activation occurs, thus inducing the expression of NF-κB-regulated genes including, in addition to the procoagulant tissue factor, adhesion molecules, such as E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1); this past may prime diabetic vasculature towards enhanced interaction with circulating monocytes. Furthermore, the incubation of endothelial cells with EN-RAGE or S100B results in VCAM-1 induction, in a RAGE-dependent manner, as sustained by the inhibitory effect of anti-RAGE IgG or soluble RAGE (Basta et al, 2004).

4. Alterations of endothelium-dependent vasodilatation

AGEs linked to the vascular matrix may quench bioavailability of nitric oxide (NO), which is an important regulator of vascular tone inducing smooth muscle cell relaxation. Studies provided that, AGE inhibits NO activity, when it added to NO in vitro. Studies on animal exterminate induced diabetes show that an alteration of endothelium-dependent dilatation occurs in short period, within 2 months, from diabetes induction. A direct reaction between NO radical and other free radicals which are formed during the reactions of

AGEs assumable leads to inactivation of NO. In parallel, advanced glyaction end products promote the expression of the potent vasoconstrictor endothelin-1 changing endothelial function towards vasoconstriction (Basta et al, 2004).

These four mechanisms promote and alter vascular wall homeostasis. When mononuclear phagocytes migrate to the site of immobilized AGEs in the tissue, their migrations allow them to interact with AGE -modified surface and become activated. This mechanism cause attracting and retaining MPs in tissue where AGEs deposit. Migration and activation of MPs and T cells (inflammatory cells) promote and cause chronic vascular inflammation through alter vessel wall (Basta et al, 2004).

Promotion of atherogenesis pathways by AGEs:

AGEs are most important factors in endothelial dysfunction in diabetic patients through binding its receptor (RAGE). Advanced Glycation Endproducts stimulate the expression of proinflammatory cells and molecules (Altman, 2003).

AGEs can be seriously deleterious to the function of blood vessel walls in various ways. First it causes blood vessel dysfunction through cross bridges among vessel macromolecules. Secondly of damage is that accumulation of AGEs lead circulating blood cells to adhere to the vascular walls which promote atherosclerosis. Third way is through binding different receptors which have been recognized on various cell types such as macrophages, endothelial cells, and smooth muscle cells, renal and neuronal cells (Basta et al, 2004).

Hyperglycaemia Mechanisms-caused damage:

Hyperglycemia diverse microvascular and macrovascular complications in diabetic patients through several mechanisms such as increased formation and accumlation of advanced glycation end-product (AGE); activation of protein kinase C (PKC) isoforms; and increased generation of reactive oxygen species (Bonke et al, 2008)

1. Increased production advanced glycation end-products

AGE promote atherogenesis pathway through accumulation of AGEs and AGEs ligation to RAGE on endothelial cells and macrophages induce inflammation through activating pro-inflammatory cytokines including TNF-α, 1L-6 a L-1ß, induction of reactive oxygen species (ROS) and through increased oxidative stress which lead to degradation of IKBs (IKBs is normally bound to NF-KB to prevent translocation of NF-KB to nucleus). ROS activates NF-KB which results in translocation of NF-KB to nucleus. Activation of NF-KB cause pathological change of gene expression which is highly related to inflammation, immunity and atherosclerosis, increased expression of inflammatory mediators which lead to insulin resistance and increased RAGE expression as well (Basta et al, 2004).

Mechanisms by which intracellular production of advanced glycation end-product (AGE) precursors damages vascular cells. Cellular functions are altered by dicarbonyl advanced glycation end products. Alteration of extracellular matrix proteins results in abnormal interactions with other matrix proteins and with integrins. Alteration of plasma proteins by AGE precursors generate ligands that bind to AGE receptors, inducing conversion of gene expression in endothelial cells, mesangial cells and macrophages.

2. Activation of protein kinase C:

The protein kinace C (PKC) family compromises at least eleven isoforms, nine are induced by the lipid second messenger diacylglycerol (DAG). Altered DAG-PKC pathway play an important role in diabetic complications. Intracellular hyperglycaemia raises the amount of DAG which activate PKC in cultured vascular cells and in the retina and renal glomeruli of diabetic animals. Hyperglycaemia may also activate PKC isoforms indirectly by AGEs bind receptors and increased activity of the polyol pathway, possibly through increased reactive oxygen species (ROS). AGE stimulated diacylglycerol (DAG) and activate protein kinase C (PKC) in VSMC. PKC is one of important signal transduction elements involved with multiple cell response. In early studies of diabetes, retinal and renal blood flow abnormalities are due to activation of PKC-ß isoforms perhaps through depressing nitric oxide production or increasing endothelin-1 activity. Abnormal activation of protein kinase C has several pathogenic consequences: It leads to decreased production of nitric oxide in smooth muscle cells and glomerular mesangial cells that is induced by hyperglycemia (Brownlee, 2001).

Activation of PKC causes inhibition of insulin-stimulated expression of mRNA for endothelial nitric oxide synthase (eNOS) in cultured endothelial cells. Hyperglycaemia activates PKC to increased endothelin-1, permeability of endothelia cells and increased expression of the vascular permeability angiogenesis factor (VEGF) in smooth muscle cells (Brownlee, 2001).

3. Increased generation of reactive oxygen species:

Increased generation of reactive oxygen species (ROS) is another possible pathways of diabetes complications especially nephropathy complication. Production of Reactive oxygen species can be result from the activation of various enzymes, including NADPH oxidase, nitric oxide (NO) synthase, and myeloperoxidase, with arising evidence that NADPH oxidase is the major cytosolic source of ROS generation in diabetes. NADPH has homologues that are present within the kidney, namely nox-3, seen in fetal kidney, and nox-4, which is predominately expressed in the renal cortex. AGE bind RAGE induces signal transduction and activate NADPH oxidase in endothelial cells. The interaction between AGE-RAGE enhances production of the cytokine vascular endothelial growth factor (VEGF), which is directly induced by NADPH oxidase and is associated with the pathogenesis of albuminuria in diabetes (Bonke et al, 2008).

Diabetes mellitus control and treatment:

Both type 1 and 2 diabetes mellitus are characterized by elevated blood glucose level due to insufficiency of insulin level. Therefore, diabetes patients need to reduce blood glucose level through healthy diet, drugs such as (hypoglycemic tablets and anti-atherosclerosis drugs), insulin injection and regular physical exercise.

1. Healthy Diet:

In general, healthy diet for diabetes patients should include: limit fats (especially saturated fats and trans-fat acid), proteins and cholesterol. Also, patient should consume a lot of fiber and vegetables. Patients with insulin-producing or insulin synthesis treatment should monitor their blood glucose

level to avoid hyperglycemia. For example, adults and teenager patients should maintain their blood glucose level between 80-120 mg/dl, and 100-200 mg/dl for children under 12 year old. Type 1 diabetes patients should test their blood glucose level four to more per day. However, in type 2 diabetes, patients recommend to measure blood glucose level 1 to 2 times daily, because glucose level in type 2 diabetes is more stable than in type 1. Such important tests are important during diet plans and treatment. For example, glycosylated hemoglobin test (HbA1 c) is an indicator for carbohydrate level. Micro and macroalbuminuria indicates of the proteinuria and renal function, for instance if the teat showed high level of proteinuria then the patient need to lowering protein intake (Flaws et al, 2002).

2. Exercise:

Physical exercise is important to manage diabetes complications. Regular exercise improves the status of both type 1 and 2 diabetes through transporting sugar to muscles, improving blood circulation, and increasing insulin receptors. Exercise has specific effects on diabetes patients include: reducing blood glucose level during and after exercise, increased insulin sensitivity, reduced triglyceride level and increased good cholesterol (HDL) (Flaws et al, 2002).

However, diabetes patients may have several risks associated with exercise which include: hypoglycemia if patient under treatment with hyperglycemia agents, hyperglycemia and ketosis in insulin-deficient patients, and exacerbation of cardiovascular disease. Therefore, patients who are over 30 year old should be examined before doing physical exercise. These examinations include: cardiovascular exams such as blood pressure, blood

lipid and ECG, and neurological evaluation such as eye exam (Flaws et al, 2002).

3. Anti-inflammatory drugs

Various drugs in recent clinical practice have been used as anti-inflammatory agents such as thiazolidinedione (TZD) class of PPARγ agonists and members of statin class of HMG CoA reductase inhibitors. Both of them have importane anti-inflammatory properities and both have action on glucose homeostasis and cholesterol redusing (Shoelson et al, 2006).

TZDs are used to induced insulin sensivity and decresed hyperglicemia in patient with type 2 diabetes mellitus. TZDs drugs inculde pioglitazone, rosiglitazone and troglitazone. The role of TZDs is through binding and activiting PPARγ to induce a number of gene expression products in a dipocyte. TZDs action are attributed to fatty acid in the a dipose tissue. TZD decreases circulating free faty acid and keep faty acid out of musle and liver because accumalte of fatty acide in these tissue lead to insulin resistance. In addition, PPARγ is not only present in dipocyte but also in it is expressed in macrophages and other immune cells response, hepatocyte, endothelial cells and vascular smooth mucsle cells (VSMC). TZD play role in reduce the expression of targets gene for cytokines, growth factors, proliferation and migration of cells, and cell cycle progression (Shoelson et al, 2006).

Rosigliatozone:

Rosigliatozone decreses the inflammatory markers such as serum C-reactive protein, metalloproteinase-9, white blood cell, tumer necrosis factor-α and serum amyloid-A in type two diabetes mellitus (Altman, 2003).

Group of studies done on Diabetes Rodents:

The recent research has demonstrated that rosiglitizone, a PPARγ agonist, attenuates diabetes associated atherosclorosis. The reaserch has studied the direct antiathersclosis effects of PPARγ afer long beriod of therapy in an experiment of insulin deficiency. This study show that rosiglitezone has no effect on glucose level in both control or diabetes mice. However, rosiglitizone , PPARγ (acting as insulin sinstiser) significally decresed plasma insulin level in control mice (Calkin et al, 2005).

In addition to Calkin study and his collegous, another study shows that rosiglitazone increase the insulin content and iselt cells number and total mass of pancreas in diabetes rats. However, it is unknown if this is adirect effect of rsiglitazone by activation of PPARγ mediated pathway in islets or indirect effect of normalizing the hyperglicemia (Sanchez et al, 2002).

Increaesd secration rate of proinsulin relative to insulin is one oa acommon feature in type 2 diabetes mellitus. The normal range of proinsulin in normal subjects is about 2 to 4% of the total amount of insulin and in type 2 diabetes proinsulin rate is about 15%. Curently the studies has been demonstrated that rosiglitazone in type 2 diabetes decrease proinsulin secretion. Proinsulin has been associated with activity of carboxypeptidase E. Fricker and collegous have evidenced that carboxypeptidase are present in secretory pathway and participate in peptide processing. CarboxypeptidaseB has similler feature as carboxypeptidaseE but the precursor sequence of carboxypeptidaseB has been seen in an adult mouse islet cells. Thus suggest that rosiglitazone may has primery effect on increasing the expression of

carboxypeptidaseB precursor protein resulting in increse proinsulin to insulin conversion (Sanchez et al, 2002).

Further more, rosiglitizone did not affect fasting insluin level in diabetic mice due to streptozotocin which induce insulin insufficency. Also, another group of study has curently demonstrated that short period of treatment with rosiglitizone decreased plaque area in diabetic mice but had no determination if effects seen were linked to insulin sensitization (Calkin et al, 2005).

More current sutdies have evedanced that rosiglitezone treatment in diabetes mice reduce gene expression of the NF-KB subunit p65. Further study has shown that rosiglitizone has no direct effect o glucose-induce upregulation of oxidative stress. Same study had investigated RAGE expression acording to preveious finding that first of all, RAGE expression increse oxidative stress and secondly rosiglitezone which used in culture media alter RAGE expression. The study had shown, the expression of RAGE gene wasn’t altered by rosiglitizone (Calkin et al, 2005).

Some study showed no significant alteretion in gene expression after TZDs treatment. This is because of almost studies were not preformed in human adipose tissue in vivo but they used adipose tissue of rodents and adipocyte cell line. Actually under such study we should consider about some facts that, for example rosiglitazone dose which used in rodents study (10 mg/kg) is higher than human dose about 100-fold. furthermore, TZD may has an action on gene expressed in rodents but it is not necessarily to has same action on human gene. For example carbonic anhydrase 3 protein raised 2-

fold after treated the mice with rosiglitazone, however there was no increasing in carbonic anhydrase 3 mRNA in human adipocyte. Although there were similarities in the action of TZD-induced insulin sensitivity, there may be TZD action has different mechanisms on experimental models and human (Kolak et al, 2007).

Therfore, I'm going to discuss about the studies which have been done on human adipose tissue and there findings.

Group of studies done in human patient with type 2 diabetes:

Overactivity of ubiquitin-proteasome system is associated with the inflammation and atherosclerotic plaques in type 2 diabetes. Study by Marfella and his colegous on human diabetes patients has been shown that the PPAR-γ agonist rosiglitazone reduce ubiquitin-proteasome activity and thuss prevent plaque progression to unstable phenotype in diabetes individulas (Marfella et al, 2006).

Also, same study shows, the production of O2- by monocytes is reduced after rosiglitazone treatment. Thus proteasome reduction is induced by inhibition of oxidative stress and polyubiquitination. As oxidative stress induce insulin resistence through NF-kB activation, rosiglitazone enhance insulin sensivity and plaque stability in diabetes patien through reducing oxidative stress and ubiquitin-proteasome activity.

Further possibility that, NF-KB activation is inhibited by rosiglitazone through a PPAR-γ independent pathway. PPAR-γ agonist inhibits NF-KB translocation and subsequent DNA binding through inhibits immune response which induce degradation of IKBs (Marfella et al, 2006).

Rosiglitazone have significant effects on metabolism of faty acid and lowering circuliting non-esterified fatty acid. Therfore rosiglitazone prevent islet cells through reducing fatty acid exposure (Sanchez et al, 2002)

Kolak and his colleagues investigated the gene of expression in human adipose tissue in vivo in type two diabetes mellitus, befor and after tratment with rosigliatozone and metformin. Reosigliatozone modulates expression of gene which involved in free faty acid synthesis and storage, protein strcture, inflammatory cells inculde macrophage and gene associated in glucose transport and insulin sensivity. Whereas metformin has no effect on these gene (Kolak et al, 2007).

TZD decrease the expression of RAGE endothelial protien.

From preveious finding, TNF-α increase the expression of RAGE on endothelial cells, so