The human body is termed as the perfect machine. It’s a perfectly designed system comprising of different sub-systems. These subsystems comprising of different organ systems work together in a complex, dynamic and efficient manner for its perfect health. Complexity is the characteristic of highly evolved human beings. Its efficiency depends on how well it adjusts to both external and internal perturbations. Randomness, evolvability, robustness and non-linearity are the key mechanisms that affect the adaptability of a biological system. Maintaining proper health is obviously one of the deciding factors for the onset of aging and age-related disorders. The neural, immune and metabolic systems are examples of highly interconnected dynamic systems responsible for the working of a perfectly healthy human body.

Aging is accompanied with the decrease in cellular functional complexity. It is described as a gradual loss of cellular homeostatic capacities (Himmelstein et al., 1990). There is a marked increase in the functional deregulation of cell signalling and decreased cell repair and remodelling. In other way it can be described as an increased tendency of the cellular state being locked up in a single state or takes more time to recover from the altered, abnormal physiological state. Moreover it is the constant accumulation of damage or perturbations that affects the cellular function and ultimately its survival. Question is, where does the aging process starts or gets initiated? It has been suggested that it’s an overall decline in the functionality of different sub-systems. As we age, the perturbation in one sub-system, starts to perpetuate and affect other sub-systems. Thus, high inter-connection and complexity which made humans an advanced species, is now seems to be responsible for the aging of the human body.

The functional complexity between different organ systems is maintained by the changes in gene expression and cell signalling networks. Constantly studied and understood is the, fact that alteration in these factors is responsible for the onset of age-related disorders. There is a decrease in gene expression for energy metabolism and increase in gene expression for cellular stress as seen in mouse model. There is also an increase in the activity of defence system. Things become worse when this alteration occurs early in life of an individual. With drastic changes in food habits and lifestyle among the young generation along with the increase in mental stress, has caused an alarming rise in the prevalence of type 2 diabetes in individuals as young as
25 years. This is a major concern as this trend can negatively affect the economic development of the country. Hence it is important to study these factors, to the cellular level.

The human body is exposed to the day to day challenges affecting the structural and functional integrity at the cellular level. Metabolic and immune sub-systems are constantly part of these challenges. The importance of the neural system cannot be ignored as it coordinates the proper functioning of all the systems. Thus these age-related diseases are best understood by studying these sub-systems together and the underlying cellular perturbations.

Increased metabolites in the body are regulated with the timely response of the body to implement homeostatic mechanisms. The efficiency of this response is robust in young and healthy individual, but gradually weakens as we age. Chronic inflammation and increased production of ROS is observed with the onset of aging. These are the underlying factors for the development of metabolic disorders. These also cause gradual impairment of the degradative processes of the cell, the reactive metabolites either produced through normal metabolism or absorbed directly through diet starts to accumulate in the body. Advanced glycation end product is examples of such reactive metabolites. They are formed by the reaction of reactive glucose metabolites such as MG and glycoaldehyde with the lysine and arginine amino acids of proteins. The process is called glycation. These are normally formed during the aging process and at an accelerated rate in hyperglycaemic conditions. Glycation results in the structural alteration of the protein, ultimately impairing its function. AGEs have been implicated in various pathologies of diabetes such as atherosclerosis, retinopathy, renal failure and neurodegeneration.

The cellular responses of AGEs are mostly mediated through their specific receptor called RAGE, which belongs to the Ig super-family class of receptors having similarities with the pattern-recognizing receptor. RAGE has a small cytoplasmic domain which is required for the downstream signalling.

This study was undertaken to understand the AGE mediated cellular responses and its underlying cell signalling pathways. In compliance with the previous studies, it was found that upon AGE treatment of cells, there is an increase in intracellular ROS
generation which is dependent on RAGE. This early response proceeds with the activation of redox-sensitive kinases such as IKKs, JNKs and ERKs. There was an increase in the activities of different transcription factors related to inflammation and oxidant stress, lipogenesis and calcium signalling. There was also a decrease in the troglitazone induced PPARγ transcriptional activity, which has a regulatory role in glucose and lipid metabolism, which is also seen to be downregulated in diabetics. The constantly active ERK kinase was found to be responsible for the downregulation of PPARγ activity. Surprisingly it was found that the ERK kinase can stimulate the activity of IKK in a positive way. This was found to be responsible for the AGE mediated activation of SREBP and lipid accumulation in cells. AGE mediated early cellular responses were abrogated with the administration of different antioxidants to the cells. These include inhibition of increased ROS and the transcription activity of NF-κB and AP-1. Anti-oxidants were effective in inhibiting this AGE-mediated response but were only partially able to block AGE-mediated ERK kinase activity. This was found to be the major factor for the AGE mediated downregulation of PPARγ and upregulation of SREBP transcriptional activity. Sustained activity of these kinases is the rate-limiting factor. This might probably implement chronic inflammation, insulin resistance and lipid accumulation in physiological conditions. What causes the impairment in the break-down of the accumulated lipids remained unanswered. In a recent study, it was observed that the oral administration of MG-derived AGEs in mice elicits insulin resistance and diabetes (Cai et al., 2012). The AGE-mediated signalling explored in this study represents the molecular mechanisms of these AGE-mediated system responses. Only mangiferin was found to restore this cellular deregulation by potently inhibiting AGE-mediated ROS and NF-κB activity along with complete inhibition of ERK and IKK kinase activity. It was also found to induce PPARγ transcriptional activity and DNA binding activity in vitro although was less potent than troglitazone. In silico, study suggested that it may act as an agonist of PPARγ by binding to the ligand binding domain. This supports the recent findings suggesting the anti-diabetic and anti obesity properties of mangiferin in animal models. Mangiferin by modulation of multiple targets, optimally restored the cellular homeostasis effectively.
Metabolic burden in humans is also associated with the onset of cognitive decline characterized by neuro-inflammation and neurodegeneration. MRI and PET scan of the brains from the elderly people with diabetes and Alzheimer’s showed the deposition of the amyloid plaques which absent from the brains of healthy and young individuals. These amyloid plaques are the precursors of AGE which are also found deposited in the diseased brains.

The late AGE-mediated cellular responses which becomes evident by 48 h, includes the induction of cell death especially in neuronal cells. The AGE-mediated cell death was not aggressive and was about 30-40% compared to the untreated cells. There was an increase in the activity of caspases 3, 8 and 9. Interestingly, the increased activity of caspase 8 suggests the induction of extrinsic pathway of apoptosis. Surprisingly, the AGE-mediated cell death was found to be independent of intracellular ROS generation which is an early AGE-mediated response and declines after 24h. Increased level of intracellular calcium has been found to promote tau protein phosphorylation and induces the accumulation of amyloid and NF-AT transcriptional activity. It was found that the increased secretion of IL-8 is responsible for the increased intracellular calcium levels resulting in the activation of NF-AT. One prominent pathological feature seen in diabetes and obesity is the infiltration of the immune cells into various organs such as liver, pancreas and brain. AGE-mediated activation of these cells can mimic the innate defence mechanism by increased degranulation of proteases and cytokines. This can damage the surrounding tissue and re-start inflammation in a feed-forward loop. This causes an increase in the FasL protein levels which ultimately induces cell death. Reports, suggest that AGE-mediated activation of NF-AT and NF-κB also increases the expression of β-secretase β APP-cleaving enzyme-1 (BACE-1), a protease which is also an underlying factor for the generation of amyloid plaques. PPARγ is a negative regulator of BACE-1 expression by directly binding to the PPARγ binding site at its promoter region. RAGE is also known to induce autophagy which was found to be protective against AGE-mediated cell death. Impairment of autophagic mechanism may be the underlying cause of neurodegeneration and obesity which need to be studies further.

RAGE-mediated cellular response prompts us to ask the probable reason for its presence in the normal physiology, although in lower amount apart from its role in
brain development in early developmental phases. We hypothesize that it can recognize AGE-like aggregates to activate inflammation which will result in the recognition and degradation of these aggregates. Thus this protective system gradually turns from friend to foe with the onset of diabetes and AD.

Diabetes and obesity are age related disorders and have complex disease phenotypes. Multiple pathways are de-regulated in these disorders. So it is better to design a therapeutic regime which is capable of bringing back the cellular homeostasis to normal. In this study, it was found that AGE-mediated early responses which include mainly chronic inflammation, oxidant stress, and downregulation of PPARγ transcriptional activity and upregulation of SREBP transcriptional activity were potently prevented by mangiferin among all the other antioxidants. It is interesting to note that mangiferin has been used as to treat diabetes and obesity since ancient times and is still in use in South America and China. Numerous reports have also suggested its potent therapeutic properties in animal models.

Taken together, the total body AGE burden increases due to increased endogenous formation and absorption which outruns the clearance and detoxification process. This initiates various cellular responses mostly mediated through RAGE by inducing different cell signalling pathways. This is now seemed to be one of the major contributing factors in the pathogenesis of diabetes, obesity and neurodegeneration. Hence this study is going to contribute to the existing knowledge of the AGE-RAGE axis and dependent cellular responses and will help in designing therapeutic regime for the holistic recovery of the cellular homeostasis.

By the combined modulation of these sub-systems and the different signalling pathways involved, a better therapeutic regime can be designed and implemented.
Fig. 6.1. Proposed model for AGE-mediated signaling.