Synopsis

The popularity of fried and processed food has increased over the years and so is the prevalence of obesity and diabetes, making it a growing medical concern. Processed foods contain AGE (Advanced Glycation Endproducts) which is responsible for deleterious inflammatory signalling which worsens health conditions in diabetes patients. Diabetic people have hyperglycemia which creates a perfect soup for elevating AGE formation. More than 75% of diabetes patients belong to developing countries, with India at the top of this chart with BMI ≥ 30 kg/m² (32.9%), age and sex standardized. Accumulation of AGE in diabetes patients aggravates the weight gain complications. Adipose tissues are energy depot and plays important role in energy homeostasis, glucose and appetite regulation, expenditure, and immunity. Deregulated metabolism often poses concerns of uncontrolled weight gain and comes with series of health complications. Obesity is the primary step to several associated ailments. It represents an abnormal accumulation of adipose tissue resulting from chronic over-nutrition and reduced physical activity and is associated with deleterious perturbations including excess fatty acid secretion, increased production of inflammatory cytokines, and abnormal adipocyte hormone signalling resulting in insulin resistance.

AGE has been shown to have role in inflammatory neuronal disorders especially, Prion’s disease where autophagy is also involved but the precise connection is still elusive. Autophagy is designed to maintain cell homeostasis and has been implicated in several disorders. Therefore, we wanted to check if autophagy works to counteract AGE induced cellular burden or if AGE itself acts as trigger to start autophagy. All the existing voids in mechanistic studies to correlate and interlink multiple pathways which may manipulate signalling cascades and harm cellular homeostasis prompted us to hypothesize if AGE induces autophagy and which signalling pathway regulate this process.

The concerns get exemplified by the observation that current drugs available for diabetes treatment are associated with several side effects including obesity. Therefore, to develop new therapeutic substitutes with least side effects, we need to understand how high sugar and AGE formation aggravates these health problems and how to reverse the effects. The present study is aimed to understand the precise role played by AGE in inducing autophagy and modulating associated signalling.

My work focuses on detecting AGE mediated autophagy and identification of key signalling molecules activated by AGE, which is responsible for regulation of autophagy and its cross
talk to various cellular processes including apoptosis and lipogenesis. We demonstrate that AGE induces autophagy independent of cell types and origins. Autophagy induction follows a RAGE-AGE axis. The upstream key regulators responsible for AGE mediated autophagy machinery are described. This study shows a pivotal role of NF-κB in autophagy which is involved in its regulation with the assistance of a complex coordinated cascade of PKC, Raf kinase and MAPK pathway. AGE promotes lipogenesis. This study focuses on finding upstream regulators of lipogenesis pathway. We found involvement of NF-κB controls lipogenesis through PKC and Raf kinase. We also tried to find connection between autophagy and lipogenesis. Surprisingly, we found that AGE compromised autophagosomes clearance in p53 negative cells. This was accompanied by accumulation of several autophagy proteins including p62, LC3B and LAMP2B. Other peculiar manifestation was cleavage of Beclin1. We observed an NF-κB dependent route for Beclin1 cleavage. We demonstrate that NF-κB targets NEDD4 mediated beclin1 cleavage in p53 negative cells which have high NF-κB expression in absence of p53. Beclin1 interacts with p53 which might be shielding it from NEDD4 interaction and ubiquitination by engaging it. We illustrate how these manifestations are representative of AGE mediated autophagy impairment in p53 negative cells. In this study, we have demonstrated a “switch mechanism” which activates apoptosis upon autophagy impairment and depends upon the interacting partners of Beclin1. Another highlight of the study is the inhibitory effect of mangiferin in AGE mediated increase in ERK phosphorylation, NF-κB and SREBP activation driven lipid accumulation.

To summarize, for the first time, we have showed major regulatory signalling mechanism of AGE mediated autophagy, autophagy impairment in p53 negative cells and induction of apoptosis. Also, we have described the mechanism of AGE mediated lipogenesis induction and role of autophagy in lipogenesis and mangiferin as suitable candidate for developing new drug compounds to treat diabetes patients.

Based on above work, we have published two articles which are-


The chapter-wise categorization of this thesis describing the findings briefly mentioned above is listed below.

**Chapter 1** summarizes the introduction of cellular homeostasis, process of autophagy and its importance in homeostasis maintenance, important genes of autophagy, AGE (Advanced
Glycation Endproducts), importance of AGE as a candidate for this study, AGE-mediated signalling, role of NF-κB, p53 and SREBP, lipogenesis, hypothesis, and objectives of the study and the brief inferences of this study.

Chapter 2 is review of literature relevant to this study. This is comprised of general autophagy machinery, autophagy inducing stimuli, positive and negative regulators of autophagy, transcriptional regulators of autophagy, pathophysiological roles of autophagy, AGE and AGE mediated signalling perpetuations, AGE formation and its external sources, role of AGE in pathophysiology and pharmacological inhibition of AGE’s deleterious effects.

Chapter 3 describes materials and methods used in the study.

Chapter 4 discusses in detail the AGE mediated signalling involved in autophagy induction and its regulation.

Chapter 5 illustrates the mechanism of AGE mediated lipogenesis, involvement of autophagy in lipid accumulation and its regulation.

Chapter 6 describes AGE mediated autophagy impairment specifically in p53 negative cells which leads to induction of apoptosis.

Chapter 7 concludes the findings of the current study and discusses the future perspectives.