Overweight and obesity are serious and increasing public health problem which reached a size of global epidemic named as "globesity". Obesity and associated diseases are classified as a civilization diseases. According to World Health Organization (WHO), 39% of adults were overweight and about 13% of the world's adult population were obese. As WHO informs in European region over 50% of people are overweight or obese. Obesity is a complex condition with serious social and psychological dimensions, that, contrary to conventional wisdom is not restricted to industrialized societies but affects both developed and developing countries. The WHO predicts that the health problems associated with overweight and obesity may soon replace traditional public health concerns such as undernutrition and infectious diseases. Because obesity is known to lead to chronic and severe medical problems including insulin resistance and type 2 diabetes (T2D) and the number of obese people is increasing at an alarming rate throughout the world, there is a need to search for new target for insulin resistance treatment. It is a great challenge to discover new targets for effective treatment of the diseases or new biomarkers that would give a diagnostic tool for detection on early stage of predisposition. Liver, besides adipose tissue and skeletal muscle, is a key player in glucose and lipid metabolism. High fat diet (HFD) and obesity are associated with a number of alterations in liver including lipid accumulation that lead to impaired glucose metabolism. In liver, insulin resistance has been linked to bioactive lipid accumulation such as: ceramide, DAG and LCACoA Because accumulation of the lipids is perceived as responsible for the development of hepatic insulin resistance, then the cellular mechanisms that lead to alterations in intracellular lipid accumulation need to be understood. There is ongoing discussion which of the above mentioned lipid species plays the most important role in fatty acids (FA)-induced hepatic insulin resistance. Therefore, the main goals that we will try to achieve are:

1. Find the critical points in insulin signaling pathway affected by particular bioactive lipids.

2. Find out what is the mechanism of intracellular bioactive lipids accumulation,

3. Find a biomarkers that would be easily used in diagnosis at early stage of insulin resistance.

To accomplish this goals we plan to perform experiments with *in vivo* silencing of genes encoding key enzymes responsible for synthesis of the particular bioactive lipids in liver of HFD-induced insulin resistant mice. Moreover, we want to gain knowledge about exact mechanisms of intracellular bioactive lipids accumulation. This can be addressed by mass-spectrometry (MS) based, stable isotope tracer techniques developed by myself at the Department of Endocrinology, Diabetes Metabolism and Nutrition, Mayo Clinic, Rochester, USA. The use of stable isotope-labeled fatty acids tracers, combined with the novel MS-based methodology will allow to measure synthesis rates of key lipid intermediates (LCA-CoA, DAGs and Cers) by simultaneous quantification of both, the concentration and isotopic enrichment in liver samples. This stable-isotope based approach will shed a new light on the fate of plasma-borne FFA within liver and will allow establishing their role in induction of hepatic insulin resistance. Taking together, silencing the expression of individual enzymes responsible for bioactive lipids de novo synthesis, together with stable isotope-based *in vivo* labeling of signaling lipids, would give us an opportunity to observe the origin and the impact of a given lipid on insulin signaling pathway. The proposed research is related to basic science with a potential of breakthrough discovery in clarification of the mechanism which lead to induction of insulin resistance state that can provided a necessary knowledge for invent new therapy for insulin resistance or type 2 diabetes. Moreover, because we are not willing to restrict our understanding of lipid-induced hepatic insulin resistance by focusing only on handful of the known target proteins but want to have a chance to discover new proteins that would be involved in induction or can be implicated in the insulin resistance, we aim to follow the changes induced by HFD and gene silencing on proteins (proteome) level. The pleiotropic changes introduced by gene silencing in insulin resistant liver can be fully described only by using high throughput techniques such as proteomics. The high throughput analysis will compare the profiles of proteins between silenced and not silenced liver, which will give us an opportunity to observe the impact of lipid re-distribution on the individual lipid group. This high throughput analysis with identification and quantification of thousands of liver protein will also elucidate the state of many signaling pathways in insulin resistant liver. Moreover, this approach can reveal signaling or metabolic pathways that have not been regarded yet as the participants in lipid – induced hepatic insulin resistance. This will allow for identification of novel diagnostic or therapeutic targets.