Non-Alcoholic Fatty Liver Disease (NAFLD) is the most prevalent liver disease, affecting approximately 24% of the worldwide population. The change of eating habits of the society allied with plummet of physical activity are considered as the main risk factors contributing to NAFLD pathology. Reversible at the level of steatotic stage, NAFLD can progress to inflammatory Non-Alcoholic Steatohepatitis (NASH) and into more severe stages: fibrosis, cirrhosis which can end with liver failure and hepatocellular carcinoma. Currently, biomarkers to stage the disease as well as effective pharmacological treatments able to treat or even delay the NAFLD are lacking. The high incidence of the disease and the urgent need for effective therapeutic strategies has driven an extensive number of fundamental and translational research. Although, it is still lacking the knowledge about the primary mechanisms involved in the progression of NAFLD to NASH. According to the literature, a pro-oxidative state has been described to be associated with altered mitochondrial structure and function during NAFLD development. Moreover, it is known that dysfunctional mitochondria could lead to higher reactive oxygen species (ROS) generation and consequently a vicious cycle of oxidative stress and oxidative damage at mitochondrial and cellular level ensues. These mechanisms seem to be key players in the early changes of NAFLD development that may cause mitochondrial dysfunction, apoptosis and inflammation which may hasten NAFLD into NASH progression. Still, at what time does the system tilts towards a point of no return, it is still unknown. Based on this, we hypothesize that a more pro-oxidative state can be the genesis of the extensive mitochondrial damage (at mitochondrial physiology, function and metabolic level) that precedes hepatic inflammation and liver failure in the NAFLD to NASH transition, with critical mitochondrial oxidative damage representing an important no-return point. Therefore, our aim is to disclose the oxidative stress in hepatocytes and the specific end-points for mitochondrial dysfunction that represent a point of no return and which drive the progression of NAFLD towards NASH in a non-reversible manner. In our studies, NAFLD and NASH will be induced with a high-fat combined with high-sucrose (HFHS) diet that closely mirrors human Western Diet (in terms of fat and sucrose content).

In our first task, we will characterize mitochondrial metabolism, bioenergetics, ROS production, the status of the antioxidant defense system as well as the presence of markers of oxidative damage in the liver of mice at different time-points of NAFLD development and its progression to NASH.

In our second task we plan to investigate whether the returning back to the standard diet and/or administration of a new class of mitochondrial-targeted antioxidants to mice with already developed NAFLD or during its progression to NASH will have beneficial effect on mitochondrial metabolism and ROS homeostasis in the liver.

A deeper insight into the primary mechanisms those are involved on the early stage of NAFLD development will allow the development of novel diagnostic procedures, which are currently lacking. Regarding the urgent need for effective therapeutic strategies able to delay NAFLD development and its progression to NASH, our study will provide novel insights of ROS modulating effects by a new class of mitochondrial targeted-antioxidants in an *in vivo* NAFLD model. At the moment there are no available therapies for NAFLD or NASH except a change in lifestyle, including diet.