Excessive consumption of foods rich in saturated fats causes unhealthy fat accumulation in the liver, which is directly related to the increase in non-alcoholic fatty liver disease (NAFLD). NAFLD comprises a spectrum of metabolic states ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), cirrhosis, and finally hepatocellular carcinoma as an ultimate consequence. This is predominantly meaningful in the context of chronic liver diseases, which affect around 844 million people worldwide, with NAFLD affecting approximately 25% of the worldwide population. Therefore, NAFLD is recognized as a severe liver pathology that is emerging as a major health concern. According to the literature, NAFLD is associated with alterations of lipid metabolism caused by the imbalance between hepatic fatty acid influx and clearance, leading to increased exposure of hepatocytes to cellular stress. Despite the lack of complete knowledge, it is assumed that an additional insult is needed for NAFLD progression towards NASH, possibly involving increased cellular oxidative stress along with the failure of cellular quality control mechanisms. We hypothesize that the development of NAFLD can be modulated by autophagy, one of the major quality control systems for the removal of oxidized molecules and damaged organelles. Additionally, cellular machineries involved in the formation of reactive oxygen species (ROS) appear to be affected in NAFLD. Failure of autophagy may cause the accumulation of dysfunctional organelles, which contributes to the induction of oxidative damage to the liver in NAFLD progression. Moreover, the prospects for reversing NAFLD are considerably diminished once NASH is well established, but up to this point it responds to both lifestyle/diet and therapeutics strategies. Still, no therapeutic consensus for the treatment of NAFLD exists at present, and lifestyle modifications (mainly calorie restriction and exercise) is a major challenge for the typical patient (overweight and/or obese), who is prone to failure. On this basis, we hypothesize that while autophagy is intimately involved in NAFLD pathogenesis, this quality-control system can be reprogrammed to reverse the disease. Therefore, our aim is to unravel the role of autophagy in NAFLD progression and to develop potential novel strategies to delay disease progression. Additionally, we aim to determine whether treatment with inducers of autophagy can increase the effectiveness of supplementation with n-3 polyunsaturated fatty acids (n-3 PUFA) towards the advanced stages of NAFLD, which is otherwise limited to the initial step in NAFLD (i.e, hepatic steatosis).

Our first task is dedicated to uncover the exact role of autophagy and the associated metabolic disturbances. We will decipher how the inhibition of autophagy in HepG2 cells may contribute to the development and the progression of steatosis and which are the main cellular pathways affected. Moreover, using the same cell model, we will evaluate whether the induction of autophagy can delay the progression of steatosis. We assume that rescuing the autophagic system will allow the proper removal of oxidized molecules and damaged organelles, as well as reduce oxidative stress and improve cellular metabolism.

In the second task of our project, we plan to investigate whether rapamycin or trigonelline, potent inducers of autophagy, may delay and/or reverse NAFLD development. This will be tested in a mouse model of exacerbated NAFLD based on high-fat (lard) feeding in a thermoneutral environment. We plan to characterize the possible beneficial effects of these compounds on cellular metabolism, fatty acid oxidation fluxes and the formation of ROS. We expect that rapamycin or trigonelline treatment of mice with exacerbated NAFLD will allow the removal of dysfunctional organelles and reduce hepatic damage by stimulating autophagy, thus preventing disease progression.

Third, using a proper mouse model of the disease, we will analyze the efficacy of various lipid forms of n-3 PUFA towards different stages of NAFLD and how this is related to changes in fatty acid metabolism, autophagy, and peroxisome function in the liver. We will also verify the usefulness of combination therapy using n-3 PUFA and an inducer of autophagy.

Due to the lack of approved pharmacological therapies, this work will be of great importance in NAFLD research. Our project can develop new research paradigms that will contribute to a better understanding of the role of autophagy in the development and progression of NAFLD, while contributing to the identification of possible strategies in the treatment of NAFLD based on a combination of autophagy inducers and n-3 PUFA supplementation.