## Abstract

Obesity is a growing problem in modern society, increasing steadily between adults and children. Adipose tissue accumulation increases the release of free fatty acids and free glycerol in plasma, promoting the accumulation of bioactive lipids in the liver and skeletal muscles. Here, the lipid droplets accumulation correlates with the development of insulin resistance and type 2 diabetes. AQPs are molecules dedicated to the flow of water and small uncharged compounds. AQP9 is a glycerol and H2O2 transporter expressed in the liver. From a preliminary proteomic analysis on human liver samples from obese ND, IGT or T2D patients we confirmed the increase of the proteins connected with lipid droplets accumulation as FABP4 and FABP5. We also saw upregulation of the proteins involved in the antioxidant response, a symptom of oxidative stress present in the liver of IGT and T2D patients. Interestingly, we noticed the upregulation of AQP9 in IGT and T2D. Thus, we developed a lipid overload model to study AQPs' role in this process. We showed that the silencing of AQP9 prevents the accumulation of lipid droplets in HepG2 cells, confirming its potential key role in preventing lipid overload. On the other side, the silencing of AQP11 is not significantly influencing the lipid droplets accumulation. We showed that the overexpression of AQP9 is inducing apoptosis in the cells under lipid overload, showing the toxic effect connected to the deregulation of AQP9 expression in HepG2 cells. The lipid overload limits the ability of the cells to transport hydrogen peroxide through aquaporins. Furthermore, the same lipid overload induces the accumulation of hydrogen peroxide in the ER, a symptom of early oxidative stress. We find also hints of the presence of a reductive shield around the ER of HepG2 cells, causing the reduction of HyPer in the region surrounding the ER. We developed two different approaches for the untargeted DIA analysis of experimental and clinical samples. The first one is based on the creation of an HpH-DDA ion library and VWW-DIA runs, a precise but time- and resource-consuming technique. The second one relies mainly on bioinformatics, with a GPF-Hybrid ion library and 40STW DIA runs. We are obtaining similar results with both techniques, but GPF-Hybrid + 40STW is more effective from the point of view of time and resources. The DIA analysis performed on HepG2 cells modulating AQP9 confirmed the essential role of AQP9 in the lipid overload, proposing it as a possible molecular target to prevent lipid-induced insulin resistance in the liver. In conclusion, we reached multiple aims in this study. On one side we elucidated the impact of AQP9 on the lipid overload and oxidative stress homeostasis in HepG2 cells. On the other side, we developed a novel LC/MS proteomic approach for the analysis of experimental and clinical samples in the aspect of hepatic lipid overload.