Civilization changes over the last decades have been associated with an increase in prevalence of various metabolic disorders related to pathological obesity and insulin resistance (IR) causing metabolic syndrome (MetS), and finally type 2 diabetes (T2D) development. IR, the inability of tissue to respond to circulating insulin, has become a serious global problem not only in human, but also in veterinary medicine. A similar disease in horses (equine metabolic syndrome, EMS) shares some of the features of human MetS including abdominal obesity, IR, resting hyperleptinemia and hyperinsulinemia, local and systemic inflammation, but differs in that laminitis. Laminitis is a highly painful and potentially devastating foot problem, which progression may cause perforation of coffin bone and require euthanasia. Unfortunately, due to its complexity, the pathogenesis of EMS is still not clear, which strongly limits the advancement of effective therapy.

Our team possess an extensive experience in investigation of adipose tissue and adipose tissue-derived mesenchymal stem cells (EqASCs) impairment in the course of EMS, but now, for the first time in the world, we aim to evaluate the role of liver dysfunction in EMS development**.** In human endocrinology, it is widely known that T2D develops only when hepatic autoregulation is impaired and hepatic glucose production exceeds the ability of muscle glucose disposal. What is more, MetS is highly related to high levels of liver enzymes, like γ-glutamyl transferase (GGT) and reduced sex-hormone binding globulin (SHBG) serum level, which is also highly correlated with hepatic lipogenesis and progression of non-alcoholic fatty liver disease (NAFLD). In initial research we showed that, similarly to human, EMS liver is characterized by increased IR, lipotoxicity (lipids accumulation in insulin-sensitive tissue like liver and skeletal muscle), excessive endoplasmic reticulum (ER) stress and increased peroxisome proliferator-activated receptor gamma (PPARγ) content, whereas decrease in SHBG and adiponectin level. Low SHBG content, along with high PPARγ level may enhance hepatic lipogenesis. Moreover, we observed increased levels of aspartate aminotransferase (AST) and GGT in serum of EMS horses. Upregulation of interleukin 6 (IL-6) and immune cells infiltration suggests increased inflammation. ER stress leads to activation of unfolded protein response (UPR), which protects cells against unfolded and/or misfolded proteins accumulation. We observed overexpression of several genes involved in UPR including inositol requiring enzyme 1α (IRE-1α) and X-box binding protein (XBP1). IRE-1α arm is well-established, and the most conserved branch of UPR. Moreover, IRE-1α is very important in defending cells against lethal consequences of ER stress. However, prolonged activation of IRE-1α pathway may promote cell apoptosis.

There is no comprehensive data regarding liver condition in EMS horses. However, due to liver-related MetS progression in human, we postulate that improving functionality of EMS horses' hepatocytes through silencing of destructive activity of IRE-1α may improve insulin sensitivity and SHBG biosynthesis. For that reason, in the present project we aim to investigate in depth the liver lipogenesis regulation at molecular level and examine the effect of IRE-1α silencing on lipid accumulation in hepatocytes. We hypothesize that IRE-1α silencing may improve SHBG biosynthesis, insulin sensitivity and viability of EMS hepatocytes. The project consist of several distinct steps encompassing basic research at the molecular level, which help to answer the question whether IRE-1α is a good drug target in metabolic syndrome treatment. Detailed characterization of EMS liver/hepatocytes is assigned to task one. In this step we aim to investigate hepatic lipotoxicity, inflammation, ER morphology, UPR, apoptosis, lipogenesis and IR markers and regulation of SHBG biosynthesis using quantitative reverse transcription-polymerase chain reaction (qRT-PCR), western blot and/or ELISA, immunofluorescence (IF), transmission electron microscopy (TEM). In the next stage we will perform IRE-1α-knockout in EMS derived hepatocytes/HepG2 ($Hep^{IRE\text{-}1\alpha/null}$) using CRISPR/Cas9 system. In order to evaluate molecular alternations caused by IRE-1α silencing we aim to induce IR by lipotoxicity using saturated fatty acids. Then, we will investigate whether IRE-1α silencing affect SHBG biosynthesis, IR, lipogenesis, ER stress and apoptosis using the following methods: qRT-PCR, western blot, IF and flowy cytometry-based system. The next step will involve induction of macrophages (RAW 264.7) polarization and co-culture with $Hep^{IRE\text{-}1\alpha/null}$. First, macrophages polarization will be induced using sodium palmitate and then RAW 264.7 cell will be carry out of co-culture with $Hep^{IRE\text{-}1\alpha/null}$. We plan to estimate inflammation response of RAW 264.7 after palmitate treatment and its effect on SHBG biosynthesis, IR, ER stress, apoptosis and hepatic lipogenesis in $Hep^{IRE\text{-}1\alpha/null}$ cells.

Realization of the project will substantially extend our knowledge in both human and veterinary endocrinology. Moreover, it will provide incredibly valuable information allowing deeper understanding of molecular events in liver in the course of metabolic syndrome.