Streszczenie w języku angielskim

The liver is the main organ regulating the metabolic processes, for which fatty acids are the fundamental energy substrate. Their normal level in hepatic cells is regulated by the following processes: 1) influx, 2) utilization, 3) export, 4) lipogenesis, and 5) β-oxidation. In the standard conditions, fatty acids transported into the cells are oxidized in the mitochondria and peroxisomes or esterified and then stored inside the cells. The enhanced and repeated fatty acid uptake and intracellular transport, which exceeds the body's energy requirements, promotes excessive lipid accumulation in lipid droplets. The increased accumulation of lipids is a major factor in the development of simple steatosis during NAFLD. In response to long-term and increased supply of fat in the diet, as a key source of polyunsaturated fatty acids, inflammatory pathways are activated, and the synthesis of pro-inflammatory lipid compounds is elevated. The arachidonic acid derivatives that intensify the pro-inflammatory signaling pathway promote the NAFLD progression to NASH. One of the objectives of this study was to determine the time points of high-fat feeding, in which inflammation occurs in a rat model of NAFLD.

Moreover, as a result of the increased bioavailability of fatty acids, accumulated lipids may be also esterified into lipotoxic intermediates, such as ceramide or sphingosine. These compounds play a vital role in intracellular signal transduction pathway. The increased accumulation of selected sphingolipid fractions disrupts glucose metabolism and weakens insulin action, leading to the development of insulin resistance. The another aim of this study was to define changes in sphingolipids content and phosphorylation state of the proteins from the insulin signaling pathway during the development of NAFLD induced by a high-fat diet. Based on the above findings, determining the time point when alterations coexist with fatty liver development is important in preventing steatosis from deterioration into irreversible changes.

The study is a necessity to find a pharmacological factor that restrict the formation of changes in NAFLD and the progression to NASH. A pharmacological factor that could potentially be used in the treatment of NAFLD is dexamethasone (DEX). This compound is a synthetic glucocorticoid that regulates the hepatic metabolism of fatty acids. DEX is commonly used in the treatment of many inflammatory diseases. Anti-inflammatory effect has resulted from a reduction in the activity of microsomal desaturases that rate-limit the synthesis of arachidonic acid, as a direct precursor of inflammatory mediators. It is also known that dexamethasone does not directly promote the synthesis of triacylglycerols (TAG) and *de novo* lipogenesis (DNL) in hepatocytes. Above mentioned effect results from the activation

of lipolysis processes in adipose tissue. Despite numerous studies, there are no reports precisely describing the influence of DEX on fatty acids transport and metabolism in HepG2 (hepatocellular carcinoma cells). The assessment of the total expression of fatty acid transporters in HepG2 exposed to dexamethasone will indicate the potential protective effect on lipid metabolism.

The studies were carried out using an *in vivo* model (the liver of rats fed a high-fat diet for 1, 2, 3, 4, and 5 weeks) and an *in vitro* model (human hepatocellular carcinoma cells exposed to dexamethasone and/or palmitate at two different time points – 16 h and 40 h). In the *in vivo* model, the content of arachidonic acid in selected lipid fractions and the content of sphingolipids were measured using the gas-liquid chromatography (GLC) and the high-performance liquid chromatography (HPLC), respectively. Additionally, the expression of proteins involved in the regulation of inflammation and sphingolipid pathways was performed using the Western Blot method. The oxidative and antioxidative parameters as well as the level of phosphorylated proteins from the insulin signaling pathway were determined using commercially available colorimetric kits, ELISA, and multiplex kits. In the *in vitro* model, the total content of lipid and sphingolipid fractions was determined by the GLC and HPLC methods, respectively. The expression of fatty acid transporters was evaluated by the Western Blot method.

The studies showed that hepatic steatosis induced by high-fat diet increased the accumulation of arachidonic acid in the liver tissue already in the first week of experimental feeding. This observation coexists with the increased expression of cyclooxygenase and lipoxygenase. Due to a decrease in the content of antioxidant enzymes and a concomitant increase in the level of lipid peroxidation products, hepatic inflammation was followed by the development of oxidative stress. Moreover, in the liver of rats fed a high-fat diet an increase in the accumulation of sphingosine, sphinganine, and ceramide was noted. Thus, it mediated the development of insulin resistance. In the *in vitro* model, there was a significant association between the exposure period and the dexamethasone-induced impact. Short-term (16 h) incubation increased the expression of intracellular fatty acid transporters (i.e. FABPpm). It was also revealed that prolonged (40 h) incubation of HepG2 cells with dexamethasone increased the activity of the anti-inflammatory n-3 polyunsaturated fatty acids pathway and increased the secretion of fatty acids into the medium while simultaneously lowering the intracellular content of diacylglycerol and triacylglycerol.

In conclusion, the high-fat diet activated the formation of inflammatory changes in the liver already in the first week, leading to an increased accumulation of the inflammatory

precursor – arachidonic acid. A high-fat feeding also caused the development of insulin resistance by increasing the accumulation of sphingosine, and this effect is sustained by increasing the concentration of sphinganine and ceramide. Moreover, exposure of HepG2 cells to dexamethasone showed a protective effect only after a prolonged incubation time by enhancing the secretion of fatty acids into the medium and activating fatty acids from the anti-inflammatory pathway.