Streszczenie w języku angielskim

Epidemiological studies on obesity and overweight indicate that these problems are significant and that they are constantly spreading, reaching the status of a global epidemic. The main causes of the growing number of obese people include improper diet and sedentary lifestyle. In recent years it has been proven that adipose tissue, in addition to storing energy, also plays an endocrine role by releasing a number of so-called insulin resistance mediators, such as free fatty acids (FFA), leptin, tumor necrosis factor (TNF-α), interleukin 6 (IL-6), adiponectin, resistin and other substances.

Higher plasma FFA concentration observed in obesity and as a result of HFD intake leads to increased fatty acid uptake by tissues involved in the regulation of glucose homeostasis (skeletal muscles, liver, cardiac muscle, pancreas). Intensified FFA inflow exceeding the oxidative capacity of the cells results in the accumulation of intracellular lipids, which negatively affects the metabolic function of these tissues.

Skeletal muscles, adipose tissue and the liver play a key role in the metabolism of glucose and lipids. Therefore, metabolic changes in these tissues contribute to the induction of insulin resistance. Due to the fact that skeletal muscles are responsible for 70–80% of total insulin-dependent glucose uptake, numerous scientists now focus on explaining the mechanism of lowering insulin sensitivity of these tissues. Initially it was assumed that intramuscular triacylglycerides (IMTG) are responsible for insulin resistance induction. Nowadays, however, researchers mainly examine biologically active lipids capable of inhibiting or activating enzymes that directly affect the insulin pathway and thus regulate the translocation of glucose transporter 4 (GLUT4) into the cell membrane. In addition to the long-chain acyl-CoA esters (LCACoA) and ceramides (Cer), these lipids also include diacylglycerols (DAG). So far, it has not been determined which of these lipids is most responsible for the development of insulin resistance and how they contribute to its occurrence.

In the light of the above, the aim of this study was to explain the role of DAG in inducing insulin resistance in skeletal muscles. In order to achieve this goal, local *in vivo* silencing of the GPAT1 gene in skeletal muscles was performed using shRNA plasmids, and the effect of this gene silencing on the accumulation of biologically active lipids as well as insulin signaling pathway in the gastrocnemius muscle of mice with HFD-induced insulin resistance was studied.

The experiment was conducted on male mice of the C57BL/6 strain (Jackson Laboratory Bar Harbor, Maine, USA). The mice were divided into the following groups (n = 8 for each group):

1. A group of mice fed a standard diet (70% carbohydrates, 10% fats, 20% proteins; Research Diets INC D12450J), in which non-silencing plasmids were administered to the gastrocnemius muscles of both legs;
2. A group of animals fed the high-fat diet (HFD) (20% carbohydrates, 60% fats and 20% proteins; Research Diets INC, D12492), which had the gene encoding GPAT1 (in the gastrocnemius muscle of one limb silenced by means of specific plasmids $HFD_{(-GPAT)}$), while the muscle of the other leg served as the control ($HFD_{(+GPAT)}$); these animals had non-silencing plasmid administered.

All mice were fed the respective diet for 8 weeks.

Two weeks before killing, the animals were subjected to an oral glucose tolerance test (OGTT) and a week later - an insulin tolerance test (IPTT).

20 minutes before euthanasia, each rodent had a bolus of deoxy-D-glucose-2[1,2-3H (N)] administered to the caudal vein, followed by an intraperitoneal injection of 0.5 U/kg insulin to measure glucose uptake through the muscle tissue as well as phosphorylation of insulin pathway proteins (under the influence of insulin). After the completion of intravenous infusion, the animals were euthanized. The gastrocnemius muscle was collected, frozen in liquid nitrogen and stored at -80 °C until assayed.

The determination of the lipid concentrations in the muscles was performed using ultra-performance liquid chromatography connected to a mass spectrometer (UHPLC/MS/MS). The level and degree of phosphorylation of insulin pathway proteins was determined using the western blot method. GPAT1 expression level in the isolated mRNA was determined using RT-PCR. Plasma insulin concentration was assayed by the ELISA technique, and glucose levels were measured with Accu-Chek Aviva glucometer.

In conclusion, the intake of the HFD leads to the induction of insulin resistance, as evidenced by increased fasting glucose and insulin levels, abnormal glucose tolerance, reduced insulin response, increased HOMA-IR and reduced glucose uptake in muscles of mice fed the HFD compared to the control group. Furthermore, the obtained results indicate that HFD intake leads to an increase in plasma FFA concentration as well as the levels of LCACoA, ceramides, DAG, TG and acylcarnitine in skeletal muscles. Elevated levels of biologically active lipids inhibit the insulin pathway, as evidenced by a decreased level and/or degree of phosphorylation of the insulin pathway proteins, resulting in reduced glucose uptake in the muscles. In our study, GPAT gene silencing in the muscles of HFD-fed animals decreased DAG and TG levels, which, in turn, boosted the activity of the insulin pathway and improved glucose uptake.