Streszczenie w języku angielskim

Background. Prolonged consumption of diet rich in fats is regarded as the major factor leading to the induction of insulin resistance (IRs) and type 2 diabetes. Data gathered thus far link excessive accumulation of intramyocellular lipids (IMCLs), such as diacylglycerol (DAG) and ceramide (Cer), with impairment of insulin signaling in skeletal muscles. Until recently, little has been known about the involvement of long-chain acyl-CoAs (LCACoA) in the accumulation of bioactive lipids and impairment of insulin action in skeletal muscle. Given the particular significance of *ACSL1* in initiating most of the metabolic pathways related to lipid synthesis, we hypothesized that modulation of *ACSL1* expression may positively affect insulin sensitivity of muscle cells by decreasing accumulation of biologically active lipids. In the light of the above, the aim of this study was to investigate the effect of local silencing of *ACSL1* gene expression in the gastrocnemius muscle on intramuscular accumulation of biologically active lipids and insulin sensitivity of muscle cells in mice C57BL/6J fed a high-fat diet. In order to achieve this goal, local *in vivo* silencing of the *ACSL1* gene in skeletal muscles was performed using plasmid shRNA electroporation in a single gastrocnemius muscle of mice C57BL/6J with HFD-induced systemic insulin resistance.

Material and Methods. All animal procedures were performed in compliance with the approval (35/2016) issued by the Local Ethical Committee for Animal Experiments, Olsztyn. The research was conducted on male C57BL/6J mice purchased from Jackson Laboratory at 6 weeks of age. After an adaptation period, male C57BL/6J mice were randomly divided into the control group (LFD, n = 8) fed a standard rodent diet and the high-fat diet group (HFD, n = 8). The mice had ad libitum access both to a control rodent diet (containing 70% carbohydrates, 20% protein and 10% fat) and a high-fat diet (containing 20% carbohydrates, 20% protein, and 60% fat; % energy). All animals were fed their appropriate diet for 8 weeks. In the 2nd week of the experiment, electroporation of the gastrocnemius muscles was performed with the appropriate plasmid in all experimental groups. In short, in the control group ($LFD_{(+ACSL1)}$), both hindlimb gastrocnemius muscles were treated with scrambled shRNA plasmid. To emphasize the sole effect of *ACSL1* silencing on skeletal muscle bioactive lipid accumulation and insulin signaling pathway and to remove effects of intergroup variability, we silenced *ACSL1* in gastrocnemius muscle of one limb of HFD-fed animals ($HFD_{(-ACSL1)}$ gastrocnemius), while

the opposite hindlimb muscle within the same animal was transfected with scrambled shRNA ($HFD_{(+ACSL1)}$). Two weeks before sacrifice, the animals were subjected to an oral glucose tolerance test (OGTT) and a week later – an insulin tolerance test (ITT). 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. 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 (LCACoA, Cer, DAG) in the muscles was performed using using high-performance liquid chromatography/tandem mass spectrometry. Insulin-stimulated glucose uptake was determined using a liquid scintillation counter. The level and degree of activation of the insulin pathway and the expression of selected proteins of lipid metabolism (IR, IRS-1, PKB / Akt, AS-160, PI3K, ACSL1, CD36, FATP-1, FABPpm, GLUT4, CPT1B, GADPH) were determined using the Western Blotting method. *ACSL1* expression level in the isolated mRNA was determined by RT-PCR.

Results. As expected, diet induced obesity resulted in induction of insulin resistance accompanied by elevated plasma fatty acid concentration, increased level of IMCL lipids along with inhibition of insulin pathway, and impaired glucose uptake in the gastrocnemius muscle. *ACSL1* silencing improved insulin sensitivity at the muscular level and simultaneously decreased the content of LCACoA (C16:0-CoA, C16:1-CoA, C18:1-CoA, C18:2-CoA i C24:0-CoA), SCACar (C4:0-Car, C6:0-Car, C8:0-Car, C10:0-Car), LCACar (C14:0-Car, C16:0-Car, C18:0-Car i C18:1-Car) zarówno Cer (C18:1-Cer i C24:1-Cer), jak i DAG (C16:0/18:0-DAG, C16:0 /18:2-DAG, C18:0/18:0-DAG). ACSL1 silencing was associated with increased phosphorylation of insulin pathway proteins, including tyrosine (Y632) IRS-1, serine (S473) Akt/PKB, serine (S588) AS160, and improved gastrocnemius glucose uptake from high-fat diet-fed mice.

Conclusion. The said results indicate, that the reduction of the intracellular LCACoA pool directly inhibits the accumulation of biologically active lipids such as Cer and DAG (acylated with 16 and 18 carbon fatty acids) as indicated by the substrate specificity of ACSL1. In addition, silencing ACSL1 reduces the content of SCACar and LCACar, which proves the important role of this enzyme in both the intramuscular lipid synthesis pathway and in the mitochondrial β-oxidation of fatty acids under high-fat diet load conditions.

Reduced intracellular DAG and Cer pools are associated with increased phosphorylation of the insulin pathway proteins and normalization of glucose uptake in gastrocnemius muscle of mice fed a high-fat diet under local ACSL1 silence. These data support the hypothesis that a change in the intracellular LCACoA concentration, and thus the concentration of ceramide and DAG, may result in positive changes in the activation of the insulin pathway in skeletal muscle. These findings suggest that tissue-specific modulation of ACSL1 could be a potential target in the treatment of insulin resistance. Further research should focus on the interactions between proteins involved in lipid synthesis, mitochondrial and peroxisomal β-oxidation to characterize the complex intermolecular interactions related to obesity and insulin resistance.