

Streszczenie w języku angielskim

Background: Skeletal muscles accounts for ~80% of insulin-stimulated glucose uptake and play a key role in lipid metabolism. Consumption of high-fat diet (HFD) may contribute to metabolic changes in muscles, including the development of insulin resistance. Studies conducted to date have indicated a major role of ceramides in the development of skeletal muscle dysfunction related to glucose metabolism. Unfortunately, the exact mechanisms by which these biologically active lipids work are not yet fully understood. In order to explore this topic further, this study tried to elucidate the role of ceramide accumulation in inducing skeletal muscle insulin resistance by silencing the gene encoding serine palmitoyltransferase (SPT) in the gastrocnemius muscle of animals with HFD-induced insulin resistance.

Material and methods: An experiment was performed with the use male laboratory strain C57BL/6 mice. The animals were randomly divided into 2 groups (n=8). The first group was fed a standard rodent diet, the second group was fed high-fat diet. Gene silencing was performed using shRNA plasmids. Gastrocnemius muscles of the both hindlimbs in animals from the first group were treated with scrambled shRNA plasmid. In mice from the fat-fed group, one hindlimb gastrocnemius muscle was treated with shRNA plasmid targeted towards *Sptlc2*, opposite hindlimb gastrocnemius muscle was treated with scrambled shRNA plasmids. In the animals, an oral glucose tolerance test and an insulin tolerance test were performed. Moreover, HOMA-IR index value was calculated. The concentration of muscle sphingolipids, diacylglycerols, triacylglycerols, acyl-CoAs, acyl-carnitines and plasma free fatty acids was measured using UHPLC/MS/MS analyses. The degree of gene silencing was determined by RT-PCR method, and the amount of SPT protein by Western blot method. The level of insulin signaling pathway activation was examined using western blot analysis.

Results: High-fat diet feeding caused in a significant increase in skeletal muscle *SPT* expression. It was observed also to an over ~ 70% increase in ceramide content. Accumulation of biologically active lipids resulted in a decrease in the level and / or degree of phosphorylation of proteins in the insulin pathway. In mice fed a high-fat diet with silenced *Sptlc2* gene in gastrocnemius muscle, a significantly lower content of ceramide was observed, without changes in the content of other lipids. Was noticed also a positive *Sptlc2* silencing effect on muscle

sensitivity to insulin. The decrease in muscle ceramide levels was accompanied by an improvement in skeletal muscle insulin sensitivity.

Conclusion: In summary, the results of the study show that HFD consumption leads to intramuscular accumulation of lipids, including ceramides. The accumulation of lipids in the muscles was accompanied by the state of insulin resistance. The observed increase in the content of biologically active lipids was accompanied by inhibition of the activity of proteins in the insulin pathway. Silencing the *Sptlc2* gene decreased the level of ceramide, and improved the muscles insulin sensitivity. The obtained results indicate that the controlling of de novo ceramide synthesis, may be a potential therapeutic target in the treatment of insulin resistance.

Keywords: insulin resistance; ceramide; skeletal muscle; gene silencing; electroporation; serine palmitoyltransferase