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Wpływ diety o zróżnicowanej zawartości wielonienasyconych kwasów tłuszczowych omega–6/omega–3 na profil proteomiczny i metabolizm wątroby myszy

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1. Summary

Dietary recommendation encourage reducing saturated fatty acids (SFA) in diet and replacing them with polyunsaturated fatty acids (PUFA) *n*–3 (omega–3) and *n*–6 (omega–6) to decrease risk of metabolic disturbances, diabetes and coronary heart disease. Moreover, excessive amounts of *n*–6 PUFA and high *n*–6/*n*–3 ratio is found in Western type diet. Increased linoleic acid (LA, 18:2*n*–6) intake could facilitate the production of oxidized LA-derived metabolites as well as product of LA conversion - arachidonic acid (AA) and its corresponding proinflammatory eicosanoids. There are some evidences the importance of a low *n*–6/*n*–3 ratio in diet for preventing chronic diseases. It could be linking with anti-inflammatory functions of linolenic acid (ALA, 18:3*n*–3) and longer chain *n*–3 fatty acids.

The aims of the study was conducting a complex analysis of mouse liver proteome profiles for identification changes in response to diets with varied ratio LA/ALA .

Sixty four eight-week-old outbred Swiss Webster male mice were divided into four groups (16 mice per group). Experimental groups of mice were fed diets rich in polyunsaturated fatty acids (~80%) with ratios of LA/ALA 14:1 (14:1 group) and 5:1 (5:1 group). Control group mice were fed a high-saturated fatty acids (77%) diet with very low content of PUFA (11%) (NKT group). These three diets contained 22% fat. Additional group of mice was fed a standard chow diet containing 2% of fat (STD group). The experiment was conducted in two periods – the first part consisted of 32 mice (eight per group) which fed diets for three months. The second set of animals contained 32 mice (eight per group) were fed for six months. All mice received food ad libitum. After three and six months of diet mice were sacrificed and livers were dissected out and blood samples were collected. Liver proteins were separated using two dimensional electrophoresis (2DE) in the 17-cm 3–10 non-linear IPG strips and 12% SDS-PAGE gels. Gels were staining using Coomassie Brilliant Blue G-250 method. The 2DE gel images were analysed using PDQuest 8.0.1^{TM} (Bio-Rad Laboratories, Inc.). Differentially regulated proteins were indicated/assessing using multiple regression model in SAS 9.4. (SAS Institute Inc., USA) and differentially expressed spots ($p<0.05$) were excised, destained and in-gel digested by trypsin and identified with mass spectrometry MALDI-TOF/TOF (ultrafleXtreme^{TM}, Bruker Daltonik GmbH) and Peptide Mass Fingerprint (PMF). Proteins were classified according their biological functions using String ver. 11. Protein-protein interactions

networks were created in Cytoscape 3.7.1 and ClueGo plug-in. Transcriptomics analysis were conducted using total RNA extracted from liver samples using AllPrep RNA/DNA/PROTEIN (Qiagen). 1.5µg of total RNA was reverse-transcribed using anchored-oligo d(T)18 primers and random hexamers. Real-time PCRs were carried out using SYBR Green I and LightCycler 480 (Roche Diagnostics GmbH).

In the present study among 118 differentially expressed protein spots, 76 spots were identified, including 63 protein spots corresponded to single proteins and 13 spots were mixtures of two or more proteins. MASCOT score was ranged from 61 to 346 with mean 154,44. Identification of 75% of spots was conducted above 97, sequence coverage above 32% and more than 11 peptides were matched.

The expression levels of 12 liver proteins were changed after three months of diet in the STD group compared to the NKT group. Relative expression levels of 6 proteins involved in HDL remodeling (ALB, APOA1) and amino acid metabolism (OAT, IVD) were increased, whereas relative expression levels of 6 proteins involved in fructose catabolism and glycolysis (KHK, TKFC) were decreased. Relative expression levels of 10 proteins were changed in the 14:1 group compared to NKT group, including the increase expression levels of 5 proteins involved in catabolic processes and redox reactions (APOA1, GLO1, IVD, PRDX6) and reduction of expression levels of 5 proteins associated with lipid metabolism (GPD1, HMGCS2). The relative expression levels of 14 proteins were changed in 5:1 compared to the NKT group. Relative expression levels of 6 proteins related to oxidative stress response and redox reactions (ACAT2, CA3, PRDX6) and reduction of expression levels of 8 proteins involved in the ATP biosynthesis processes (ATP5F1B, GALK1, TKFC). After 3 month of diet supplemented with polyunsaturated fatty acids with a ratio LA / ALA 14: 1 and 5: 1, the levels of peroxidoxin 6 (PRDX6) were elevated compared to the group fed with high content of saturated fatty acids diet. Increased levels of apolipoprotein A1 (APOA1) associated with reverse cholesterol transport were observed in group fed with higher LA/ALA (14:1) diet compared to 5:1 LA/ALA diet.

After 6 months of in the 14:1 group, the expression levels of 16 protein were markedly changed, including decreased level of proteins involved in amino acid metabolism (HGD, FTCD, OAT). Changes in expression levels of 10 proteins were found in 5:1 group compared to NKT group - increased expression levels of 2 proteins involved in the metabolism of reactive oxygen species (PRDX4 and PRDX6) and decreased levels of 9 proteins associated with carbohydrate metabolism (FBP1, ENO1,

GALK1). The most statistically significant difference was noted between NKT and STD groups - 21 proteins were found significantly different. In the NKT group expression of 13 proteins associated with glycolysis and gluconeogenesis (ENO1, FBP1, KHK, TKFC) were elevated and nine proteins associated with HDL transformations and methionine metabolism (ALB, APOA1, BHMT) were reduced in NKT compared to STD group. In the group fed with 14:1 diet, the expression levels of 10 proteins involved in gluconeogenesis and glycolysis (ALDH2, FBP1) and non-alcoholic fatty liver (COX6A1, EIF2S1) were significantly reduced compared to 5:1 group.

Transcriptomic analysis examined the expression levels of genes involved in lipid metabolism (*Srebf1, Fasn, Fads1, Fads2*) and genes encoding proteins which showed changes at the protein level (*Ces1d, Fbp1, Prdx6, Oat*). Increased expression of the transcription factor SREBP-1c and its downstream target – fatty acid synthase (FASN) were found to be increased in the NKT group, 14:1, 5:1 after three and six months of supplementation. These results may suggests the induction of *de novo* stimulation of lipogenesis induced by a high fat diet compared to the low fat STD group. In addition, increased relative expression of genes coding Δ5 (*Fads1*) and Δ6 (*Fads2*) desaturases were observed in a diet with a high PUFA content compared to the STD group.

Comparative analysis of 2DE gels, statistical analysis and identification of proteins using MALDI-TOF/TOF mass spectrometry showed changes in the liver proteome in response to a diet with different content of n–6 to n–3 polyunsaturated fatty acids. The study shed light on liver protein profiles of *M. musculus* after substituting SFA by PUFA. Addition of polyunsaturated fatty acids in diet caused significant changes in the relative level of liver proteins involved in lipid and carbohydrate metabolism, amino acid biosynthesis and degradation and regulation of redox homeostasis. The composition of the diet, especially the content and type of fatty acids is a factor which significantly affects the level of functional liver proteins which in consequence can influence the functioning of the body..