Summary

Fatty liver is the most common disorder of this organ. One of the key nutrients that contributes to the proper functioning of the liver is choline. Phosphatidylcholine, one of the forms of choline, plays an important role in lipid metabolism and prevents accumulation of lipids in the liver. The effectiveness of endogenous phosphatidylcholine synthesis is dependent on among others on the PEMT enzyme coded by the *PEMT* gene, whose activity depends on estrogen levels. So far, the effects of phytoestrogens, which are structurally similar to estrogens, on endogenous phosphatidylcholine production and liver function have not been investigated. However, it has been suggested that soybean intake can have a positive effect on lipid metabolism in the liver.

The aim of this dissertation was to assess effects of selected phytoestrogens, i.e. soy isoflavones on endogenous choline synthesis and lipid metabolism in rats, both in a state of choline deficiency in the diet and proper supply.

An in vivo experiment was conducted on 80 male outbred Wistar rats aged 8 weeks. In the first stage of the study, the animals were assigned to two equal groups of 40 animals each. In this part of the experiment, which lasted 4 weeks, one group was fed with an AIN-93M diet with normal choline content (CD), and the other was fed with a choline-deficient diet (CholDD). In the next stage, both groups were divided into 4 subgroups of 10 animals each, which were fed the same diet as in the first stage, but with the addition of soy isoflavones, i.e. genistein (CD-GEN or CholDD-GEN), daidzein (CD-DAI or CholDD-DAI) at a dose of 0.5 g/kg of diet or ready extract of isoflavones from soybeans (CD-ISO or CholDD-ISO) at a dose of 2 g/kg of diet. During the experiment, weekly body weight measurements were performed and also body composition analysis using nuclear magnetic resonance was performed three times. At the end of the experiment, in the biological material obtained from the animals the concentrations of selected biochemical blood parameters were measured using colorimetric or enzyme-linked immunosorbent tests, i.e. glucose, total cholesterol, LDL, HDL and VLDL cholesterol, triacylglycerols, liver enzymes, and the level of phosphatidylcholine in the collected liver fragment. Morphological assessment of liver sections was also performed and the fat content in feces collected in the last week of the experiment was analyzed according to the Soxhlet method. The analysis of the expression of selected genes (*Pemt, Ppar γ, Srebp-1c, Fasn*) in the liver at the transcription level was performed using the *real-time* PCR method, while

the PEMT, PPARγ, SREBP-1c and FAS proteins were analyzed using the Western Blot or ELISA method.

The consumption of soy isoflavones did not affect the expression of the *Pemt* gene or phosphatidylcholine content in the liver of rats, as statistically significant differences between groups were observed only in relation to the different choline content in the diet. However, there were differences in the expression of levels of *Srebp-1c* and *Fasn* genes related to lipid metabolism between groups taking different soy phytoestrogens. However, due to discrepancies in the transcript and protein abundance, this effect seems to be ambiguous. Differences in the expression of levels of the *Pemt*, *Ppar γ*, *Srebp-1c*, and *Fasn* genes were observed when comparing groups consuming proper amounts of choline in the diet compared to the group consuming food with a deficit of this nutrient. Dietary choline deficiency significantly increased *Pemt* gene expression at the transcriptional level. Nevertheless, this effect was not detectable at the PEMT protein level. Choline deficiency in a diet also led to changes in the blood biochemical parameters. Differences were noticed between the rat groups in the concentrations of total cholesterol and LDL and VLDL cholesterol in blood serum. Among the groups consuming the choline-deficient diet, mild morphological changes were also observed in hepatocytes, including the accumulation of small lipid droplets and enlargement of cell circumference. No changes in fecal fat content were observed between the groups, regardless of the content of choline and soy isoflavones in diets.

To sum up, the soy phytoestrogens, despite their structural similarity to estrogens, do not increase endogenous choline synthesis. Their impact on pathways related to lipid metabolism requires further research. However, the negative relationship between dietary choline deficiency and liver function was confirmed - the 12-week period of deficiency caused changes in liver morphology visible in the microscopic image and also influenced some parameters of the lipid profile. The obtained results confirm previous research and are another reason to create dietary recommendations focusing on the appropriate choline intake in a diet.

Key words: endogenous choline synthesis, soy isoflavones, fatty liver, gene expression, lipid metabolism

Ewelina Żuk
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