1. Research project objectives/Research hypothesis

Accumulation of fat in the liver (fatty liver) is a growing public health problem worldwide. Numerous studies have revealed that soy isoflavones can protect against fatty liver, but the mechanisms are not fully described. Fatty liver can be caused by different factors. They include for example disturbances in the so called one-carbon metabolism which consists of methionine, folate, and choline pathways. Lack of phosphatidylcholine, which is one of the forms of choline, impairs lipid export from the liver. Choline is a nutrient which can be derived from food (mostly of animal origin), but it can also be synthesized in the liver by an enzyme called phosphatidylethanolamine methyltransferase (PEMT). The rate of endogenous phosphatidylcholine synthesis depends on the expression level of the *PEMT* gene, which is regulated by estrogen concentrations. Main soy isoflavones, for example genistein and daidzein, are structurally similar to human estrogens and for this reason they can act in cells similarly to them. Taking together, we hypothesized that soy isoflavones may stimulate lipid export from the liver because they can activate the *PEMT* gene or other genes which regulate lipid synthesis and metabolism, namely *AR*, *FAS*, *PPARG*, and *SREBP1C*. The general aim of the study is thus to test the effect of soy isoflavones on liver functioning in the aspect of lipid accumulation. Specific aims of the study include:

1. Testing whether genistein, daidzein or soy isoflavone isolate can activate transcription of the *PEMT* gene, but also of other genes which are involved in fat accumulation (*AR*, *FAS*, *PPARG*, and *SREBP1C*) in primary rat hepatocytes.
2. Testing whether adding genistein, daidzein or soy isoflavone isolate to the standard rat diet can affect choline synthesis in the liver via *PEMT* activation.
3. Testing whether adding genistein, daidzein or soy isoflavone isolate to the standard rat diet can affect lipid accumulation in the liver through activation of *PEMT*, but also *AR*, *FAS*, *PPARG*, and *SREBP1C*.

2. Research project methodology

To analyze mechanisms of isoflavones activity we will perform an in vitro experiment with the use of primary rat hepatocytes (Sprague-Dawley, male). Genistein, daidzein and soy isoflavone isolate will be added to cell cultures. Expression of the *PEMT*, *AR*, *FAS*, *PPARG*, and *SREBP1C*, as well as phosphatidylcholine concentrations will be measured before and after adding phytoestrogens. To test how isoflavones act in the whole body we will perform an animal experiment. Eighty 8-week old male Wistar rats will be fed a choline deficient diet or a control diet for 4 weeks and then each group will be divided into 4 subgroups: one with the same diet, one with the diet with added genistein, one with the diet with added daidzein, one with the diet with added soy isoflavone isolate. This step will last for 8 weeks. The following analysis will allow us to describe how isoflavones affect endogenous phosphatidylcholine synthesis and accumulation of fat in the liver:

- measurements of gene transcription levels of *PEMT*, *AR*, *FAS*, *PPARG*, and *SREBP1C* genes,
- to define metabolic features of the animals biochemical biomarkers will be measured: fasting glucose, total cholesterol, HDL and LDL cholesterol, triglycerides, liver and plasma phosphatidylcholine, and blood VLDL concentrations (ELISA); the fat content of fecal samples will also be measured (Soxtec system),
- histological analysis of the liver.

3. Expected impact of the research project on the development of science

Because consumption of soy and soy products has been growing recently, studies on their impact on human health are fully justified. Previous studies have shown that soy can protect against fatty liver, but the mechanisms are unknown. The ideas proposed in this project have never been tested. Results of this study may thus increase basic knowledge on metabolism, specifically on choline synthesis regulation and lipid metabolism in the liver. Intake of soy products but also soy supplements is increasing. On the other hand, prevalence of fatty liver disease is high all over the world. For these reasons, the present study may also be of high practical importance.