Summary

Type 2 diabetes mellitus (T2DM) is considered one of the most dangerous civilization diseases of the 21st century. Often in the initial stage, this disease is asymptomatic, making it early detection challenging. Chronic hyperglycemia leads to various micro– and macrovascular complications that may contribute to multi-organ damage. Due to the continuous increase in the number of people with T2DM, it is reasonable to search for new solutions to understand the mechanisms responsible for the development of this disease. Recently, much attention has been paid to metabolomic studies, which are used, among others, to study the impact of various factors responsible for the development of T2DM.

The intestinal microbiome plays a significant role in the etiology of T2DM. It is responsible, among others, for transforming many small molecule compounds, known as microbiotadependent metabolites (MDMs), which can also penetrate the bloodstream. Other risk factors for T2DM development, such as unhealthy diet, sedentary lifestyle, and genetic polymorphisms (e.g. in the PROX 1 gene, Prospero Homeobox 1), may also cause changes in the level of MDMs. Therefore, the determination of such compounds in patients at riskcan indicate the biochemical processes leading to the development of T2DM. Gas chromatography coupled to mass spectrometry (GC–MS) is often used in metabolomics studies on T2DM. Among other applications, this technique has gained great popularity due to its ability to analyze many MDMs belonging to different classes. However, determining the widest possible spectrum of MDMs in blood samples requires optimizing the sample preparation protocol. This is particularly important in the case of GC–MS analysis, as many metabolites require derivatization before determination by this technique.

The main goal of my research was to search for metabolites related to gut microflora indicating the risk of T2DM development. To achieve the primary goal, the following intermediate objectives were set: i) the development of a serum/plasma sample preparation method for GC-MS analysis allowing for the best measurement of selected MDMs, ii) the application of the developed GC-MS method to the analysis of clinical samples to indicate differences in serum metabolic profiles between patients with prediabetes and T2DM, iii) using the GC-MS technique to determine postprandial (high-carbohydrate (HC) and normocarbohydrate (NC) meal) changes in serum metabolite profiles of healthy men depending on the presence of the PROX1 gene single nucleotide polymorphism.

To achieve the goals and verify the hypotheses, scientific research was conducted on archival biological material (serum and plasma samples) collected from Caucasian volunteers during the implementation of the project entitled "The Role of Behavioral, Anthropometric, and Molecular

Factors in the Development of Type 2 Diabetes - the 1000PLUS Project". The material obtained from 42 pre-diabetic patients (53.0±9.1 years) was analyzed. From this group, after 5 years of observation, 24 patients developed T2DM, while 18 patients remained prediabetic.

Additionally, material collected from 18 healthy men (35.5±8.0 years) was analyzed. Among them, 12 had a genetic polymorphism in the PROX1 gene promoting T2DM development, and 6 had a protective genotype. In both studies, individuals in the groups being compared were matched for clinical and anthropometric parameters. Blood from healthy individuals was used to develop the sample preparation method.

Based on the literature review, it was found that the development of T2DM is accompanied by changes in the level of metabolites related to the gut microbiota. These metabolites are mainly carbohydrates, amino acids, and fatty acids. During the optimization of the sample preparation method for determining MDMs with GC-MS, it was found that the best results are obtained when samples are prepared with methanol and water. In addition, it was shown that the volume and concentration of the methoxyamine reagent have the greatest influence on the repeatability and intensity of the measured metabolites, while the conditions of the derivatization process have the greatest influence on the completeness of this process. The developed method allows the reproducible measurement of 75 serum/plasma metabolites associated with gut microbiota. Based on the analysis of clinical samples, differences in the profile of gut microbiota-related metabolites were shown between individuals with a prediabetic state and those with diagnosed T2DM. Additionally, for selected variables ROC curve analyses were performed. It allowed the selection of seven metabolites which measurement in serum allows to distinguish individuals with pre-diabetes from those with T2DM. Both provocative tests (WW, NW) caused changes in the level of MDMs in healthy individuals with genetic predispositions for the development of T2DM in the PROX1 gene, which were not observed in individuals with a protective genotype. These differences in postprandial response may be a source of early metabolic disturbances, also at the level of metabolites related to the gut microbiota, which may be associated with future T2DM development.