# Practical analytical approach for the identification of biomarker candidates in prediabetic state based upon metabonomic study by ultra-performance liquid chromatography coupled to electrospray ionization time-of-flight mass spectrometry

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The number of diabetic patients has recently been increasing worldwide. Thus, the discovery of potential diabetic biomarker(s), leading to the early detection and/or prevention of diabetes mellitus, is strongly required. The diagnosis of the prediabetic state in humans is a very difficult issue because of the lifestyle differences in each person and ethical consideration. Based upon these considerations, animal experiments using ddY strain mice (ddY-H), which undergo naturally occurring diabetes along with age, were carried out in this study. Biomarker discovery based upon a metabonome study is now quite common, the same as that in the proteome analysis. Reversed-phase liquid chromatography-mass spectrometry (LC-MS) has mainly been used for the extensive analysis of low-molecular mass compounds including metabolites. The metabolites in the plasma of diabetic mice (ddY-H) and normal mice (ddY-L) were exhaustively separated and detected by ultra-performance liquid chromatography along with electrospray ionization time-of-flight mass spectrometry (UPLC-ESI-TOF-MS) using T3-C18 and HS-F5 columns. The biomarker candidates related to diabetes mellitus were extracted from the metabolite profiling of ddY-H and ddY-L at 5, 9 13, and 20 weeks old using a multivariate statistical analysis such as orthogonal partial least-squares-discriminant analysis (OPLS-DA). Various metabolites and unknown compounds were detected as biomarker candidates related to diabetic mellitus. Furthermore, the concentration of several metabolites on Lysine biosynthesis and Lysine degradation pathways were remarkably changed between the 9-week old ddY-H and ddY-L mice. Because a couple of biomarker candidates related to the prediabetic state were identified using the present approach, the metabolite profiling study could be helpful for understanding the abnormal state of various diseases.