Research protocol

The study population hopes to recruit about 400 participants: 200 diagnosed T2D patients and 200 healthy subjects as control subjects. All cases will recruit from T2D individuals diagnosed in the Second Hospital of Anhui Medical University, Anhui Provincial Hospital/The First Affiliated Hospital of USTC and the First Affiliated Hospital of Anhui Medical University. At the same time, we will recruit control subjects from the general population undergoing routine health examination in the same hospital.

The World Health Organization recommended the diagnostic criteria of T2D in 1999, including FPG and 2-h OGTT (75g of glucose). Patients with T2D were diagnosed because of fasting blood glucose⩾ 7mmol/L or 2-h postprandial blood glucose⩾11.1mmol/l, and we require that the standard test of these patients be completed within nearly 7 days and diagnosed as T2D by the attending physician. The T2D patients group are allowed to suffer from other diabetic nephropathy or cardiovascular diseases. The healthy people group is required to have no neurological, endocrine or other systemic diseases. And control subjects with acute and chronic inflammatory or infectious diseases will be excluded from the study.

All participants will not to exercise, fast or take a ketogenic diet before collecting urine samples. Random urine samples from these participants were collected into urine test tubes for testing.

Random urine samples collected from participants will be measured within 8 hours. Take 2ml urine out of the urine test tube and put it into a 250ml headspace bottle, seal it and put the bottle at 38℃ for 40min. These experimental parameters are obtained from a series of pre-experiments. After 40 mins, the volatile solute in the solution in the headspace bottle reached equilibrium in the gas phase, and the headspace gas of urine was directly injected into PTR-MS for detection.

PTR-MS is an on-line mass spectrometry technology based on proton transfer reaction ionization. It can calculate the absolute concentration of VOCs directly without quantitative measurement. Moreover, PTR-MS does not need to enrich the samples, and has good sensitivity and detection limit. We detect acetone in urine headspace by self-made PTR-MS. The instrument will be placed in the hospital ward for detection to prevent errors caused by long-term transportation and storage of samples.

Statistical analysis plan

The orthogonal partial least squares discriminant analysis (OPLS-DA) is used for multicomponent discriminant analysis of ions measured by PTR-MS, and the discriminant model is established. The permutation testing (with 200 permutations) is performed for the OPLS-DA model to prevent overfitting. The discrimination accuracy of multicomponent model and variable importance in projection (VIP) of each ion are obtained, and the contribution of acetone in multicomponent discriminant analysis is considered. At the same time, receiver operating characteristic (ROC) analysis was made for the discrimination score of acetone single component, and the discrimination ability of acetone single component to T2D was considered by the area under the curve (AUC). Finally, a single component of acetone is used to distinguish T2D patients group and healthy people group, in order to obtain the highest accuracy (the average value of true positive rate and true negative rate). In this way, the specific detector ion counts of acetone single component is obtained, and the determination threshold or concentration are determined.