Metabolic profiles of urine and serum from type 2 diabetic mice detected by ^{1}H NMR spectra

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Introduction

Type 2 diabetes mellitus (T2DM) characterized by the resistance of body tissues to the actions of insulin on glucose uptake has been considered as one of the main threats to human health in the 21st century [1]. It is a general metabolic disorder rather than an isolated disease entity so that detections of the whole endogenous metabolic variations with the progression of T2DM will be highly valuable. NMR-based metabonomics has been widely used to measure the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli [2]. In this work, this method was applied to investigate the urinary and haemal metabolic fingerprints of an animal model for T2DM at an early insulin resistance stage and possible biomarkers were identified and analyzed.

Materials and Methods

Five male C57BLKS/J-db/db mice were used as T2DM models while six male C56BLKS/J-db/m mice were used as control. The animal room was under controlled condition (temperature, humidity, and a 12-hour light-dark cycle) and the animals were provided with food and water *at libitum*. Overnight (24 h) urine and fasting serum sample were collected by 8 weeks old. The urine samples were then centrifuged (3000rpm, 5min at 4°C) to remove the particulate contaminants and stored frozen at -80°C until measured, while the serum samples were centrifuged (3000rpm, 10min at 4°C) and stored frozen at -80°C until NMR experiment.

In NMR experiments, a sample volume of 400μl was mixed with 200μl phosphate buffer solution (0.2M Na_2HPO_4/0.2M NaH_2PO_4, pH7.4, 100%D_2O) to minimize variations in pH. 0.3mM DSS (2,2-dimethyl-2-silapentane-5-sulfonic acid) was used as an internal reference standard at δ 0.00. ^{1}H NMR spectra of both urine and serum samples were acquired on Varian NMR System 500 MHz spectrometer at 300K. All NMR spectra were phased, baseline corrected and segmented into regions of δ 0.04 width in the region of δ 0.2 ~ 9.8. The water and urea region in δ 4.6 ~ 6.0 was excluded for urine spectra prior to pattern recognition analysis, whereas only the water region in δ 4.6 ~ 5.0 was excluded for serum spectra. The remaining spectral segments were scaled to total integrated area of each spectrum to account for the differences in concentration. Principal components analysis (PCA) was carried out using the program written by ourselves.

Results and Discussion

The score plot of PCA in Fig. 1(a) shows clear separation of diabetic and control group, which indicates the existence of discrepancy between these two classes. The location of the data points of the diabetic group is more dispersed than that of the control group, possibly due to larger difference in metabolic profiles caused by complicated metabolic pathways for diabetes pathogenesis. A number of alterations were identified from the urine samples of *db/db* mice relative to control group according to the loading plot in Fig. 1(b). The increase of alanine may be associated with a reduced insulin-mediated suppression of plasma amino acid concentrations and also be indicative of an initial tubular damage. The elevated acetate has proved to be the expression of overt renal insufficiency. An elevation of taurine level is considered as the most important marker for liver damage caused by abnormal glucose metabolism. The increased hippurate reflects difference in gut microfluoral population. The higher citrate excretion may depend on increased citrate production in tubular cells and/or on reduced citrate re-absorption of tubular fluid due to glucose flow. The over-excreted TMAO may be linked to the hyperosmotic effect of glucose and be indicative of a papillary dysfunction. Besides, glucose overflow which stimulates some glycolytic enzymatic activities in tubular cells may also account for the changes in succinate and pyruvate. The different urinary excretion of creatinine, butyrate, glycine and arginine were also observed and further studies are in progress.

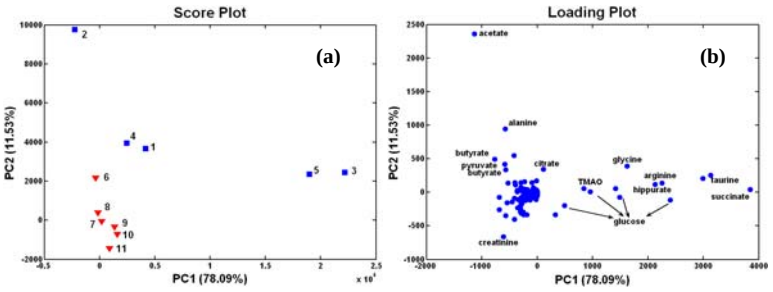

Fig. 1 PC score plot (a) and loading plot (b) (PC1 vs PC2) of ^{1}H NMR spectra of urine from db/db (1-5,■) and db/m (6-11,▼) mice.

Figure 2(a) shows the metabonomics differences of serum samples between the *db/db* and *db/m* groups, where the separation of two classes is obvious in despite of the individual differences in either class. The loading plot illuminates that this separation is attributed to the metabolites with chemical shifts of δ 0.97 (leucine, valine), 1.20 (3-hydroxybutyrate), 1.33 (lactate), 2.36 (glutamate), 2.41 (glutamine) and 3.21 (choline). Elevated plasma lactate concentration was reported in high-fat diet induced insulin resistance model mice and may reflect the effect of hyperglycaemia on enhanced muscle lactate production. Significantly increased level of 3-hydroxybutyrate mainly from elevated hepatic synthesis were found in the hyperketonemic diabetic animals. Glutamate and glutamine, intermediates and products of the tricarboxylic acid (TCA) cycle may alter with the effect of 3-hydroxybutyrate-derived acetyl-CoA. Leucine and valine were found depressed evidently in diabetic mice, analogous to the previous findings in humans which were taken as one of the biomarkers for T2DM. In addition, the decreasing choline was recognized, identical to the result from the horse blood of insulin resistance subject [3].

Conclusion

In this study, high resolution ^{1}H NMR spectroscopy integrated with multivariate statistical analysis was applied to investigate the urine and serum of T2DM mice. Clear differences between 8-weeks-old diabetic (db/db) and control (db/m) mice were observed in both urine and serum samples and a number of characteristic metabolites contributed to class separation were identified and analyzed. These results illustrate the significant alterations in metabolic profiles of urine and serum at the insulin resistance stage of T2DM animals and the metabonomics method has a potential in detecting the biomarkers for T2DM and discovering the mechanism of the disease.

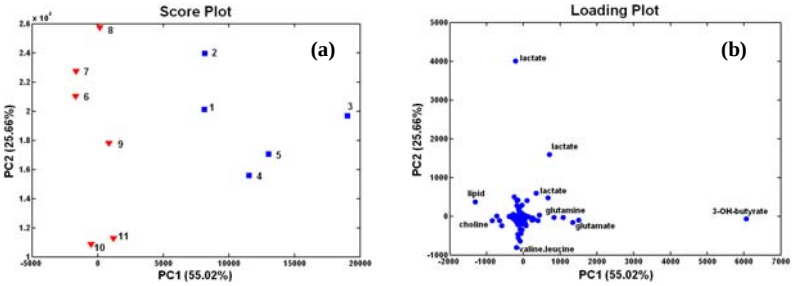

Fig. 2 PC score plot (a) and loading plot (b) (PC1 vs PC2) of ^{1}H NMR spectra of serum from db/db (1-5,■) and db/m (6-11,▼) mice.

Acknowledgements

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References

[1] Zimmet P, et al. Nature 414 (2001) 782-787.
[2] Nicholson JK, et al. Nature 1 (2002) 153-161.
[3] Hodavance MS, et al. Anal Bioanal Chem 387 (2007) 533-537.