Metabolic profilings of urine from high fat-fed rats based on ^{1}H NMR metabolomics

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Introduction

Obesity, a pre-disease state of metabolic syndrome, has been associated with not only multiple genes but also environmental factors including diet. In this study we built up a highly controlled experimental model by dietary intervention and studied the metabolic variation with the progress of obesity and insulin resistance. The metabolic differences of chow- and high fat-fed rats were analyzed by NMR-based metabolomics [1]. The results help to characterize and understand the biochemical signatures of obesity and insulin resistance.

Materials and Methods

Animals were maintained in a humidity-controlled room with a 12-h light-dark cycle. Male Sprague–Dawley littermates (8 weeks-old) were randomly segregated into two groups who received either chow or high-fat diet [2] for 22 weeks. Food and water was available *ad libitum* throughout the feeding course. Urine samples were collected from chow-fed (control, n = 11) and high fat-fed (treatment, n = 11) rats after 5, 16 and 21 weeks of feeding. One high fat-fed rat died before the 16 weeks collection. Samples were centrifuged (3000rpm, 5min at 4°C) to remove particulate contaminants and then stored at -20°C prior to NMR experiment.

Phosphate buffer solution (0.2M Na_2HPO_4/NaH_2PO_4, pH 7.4, 99.9% D_2O) was added to minimize variations in the pH of the urine samples and 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 urine samples were acquired on Varian NMR System 500 MHz spectrometer at 298K using a standard presaturation pulse sequence for water suppression. 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 region of water and urea (δ 4.6 ~ 6.0) was excluded prior to partial least squares discriminant analysis (PLS-DA). The data were normalized to the sum of spectral integral to account for the differences between urinary concentrations.

Results and Discussion

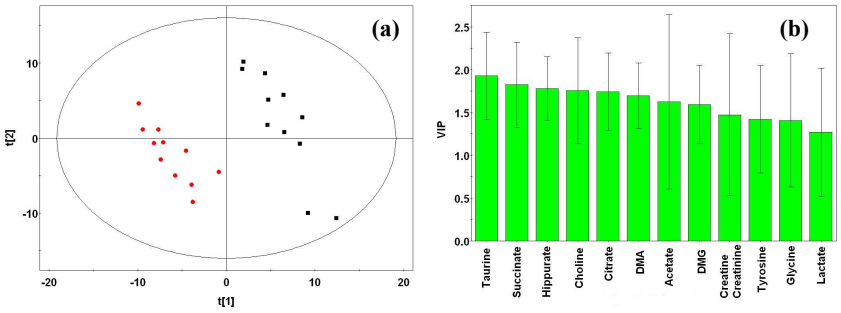

Fig. 1 (a) PLS-DA scores plot of the urine samples from 5 weeks of chow- (■) and high fat-fed (●) rats. (b) Plot of variable importance in projection (VIP) generated from (a) showing the relative contribution of each metabolite to classification.

Figure 1(a) shows the PLS-DA scores plot of the urine samples after 5 weeks of high-fat feeding. Validation of this regression model produces R^2 = 0.955 and Q^2 = 0.902, which suggests the model to be robust for biological interpretation. After 5 weeks of feeding, there was obvious insulin resistance on the high fat-fed rats. Although the spectral changes relative to the control ones are very subtle by visual comparison (data not shown), samples from the chow- and high fat-fed rats are separated from each other in Fig. 1(a), indicating the existence of metabolic differences. The plot of variable importance in projection (VIP), a measure of metabolites contributed to discriminating the chow and high fat-fed animals (Fig. 1(b)), shows the concentration alteration of taurine, succinate, hippurate, choline, citrate, dimethylamine (DMA), acetate, dimethylglycine (DMG), creatine, creatinine, tyrosine, glycine and lactate. These metabolites are mostly involved in energy metabolism and may be representative of the perturbations with insulin resistance. Glycine, a key metabolite in nucleic acid synthesis, is elevated with high-fat feeding compared to the controls, which has been attributed to the change in energy status. Increase of citrate with high-fat feeding has been proved to be a consequence of hyperglycaemia, insulin resistance, a heavy reliance on fatty acid utilization and a decreased liver clearance.

The PLS-DA was also carried out to identify the metabolites most directly responsive to the development of obesity after 5-, 16- and 21-weeks of high-fat feeding (Fig. 2). Although the data points are located dispersedly due to individual differences and random noises, three groups are clearly distinguished in the scores plot. The most significant metabolites for classification include amino acids, such as glycine and phenylalanine, and energy metabolites, such as citrate, acetate and lactate.

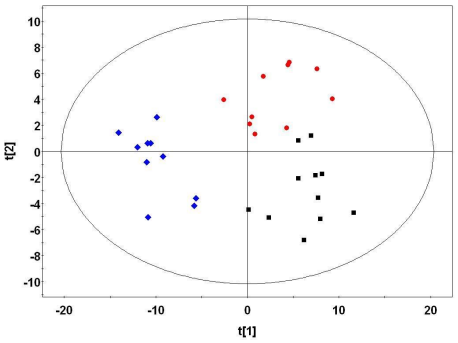

Fig. 2 PLS-DA scores plot of the urine samples from 5 weeks (■), 16 weeks (●) and 21weeks (◆) of high fat-fed rats.

Conclusion

The metabolic profilings of urine from chow- and high fat-fed rats were found to differ significantly based on ^{1}H NMR spectroscopy and PLS-DA. This study is helpful to the investigation of pre-disease state of metabolic syndrome and related intervention therapies.

Acknowledgement

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References

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