Metabonomic differentiation of long-term dietary intervention with NMR spectroscopy of human urine samples

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Introduction

Dietary composition has been proved to influence the metabolic phenotype and be related to prevalence and risk for several chronic diseases [1]. Vegetarian diet characterized as "plant-based diet" is thought to benefit body health. In this study, the variations in metabolic phenotype in urine of human participants following vegetarian and omnivorous diets are investigated using NMR-based metabonomics approach to see the diet effects from the perspective of systems biology.

Materials and Methods

25 monks from Nan Putuo Temple in Xiamen and 25 omnivorous men (control group) were studied. These vegetarians lived in a self-contained community and usually ate meals from a communal kitchen where the food was prepared strictly according to vegetarian principles. Overnight urine samples were collected from all subjects and centrifuged (3000rpm, 5min at 4°C) to remove the particulate contaminants and then stored at -20°C freezer until NMR measurements.

Phosphate buffer solution (0.2M Na_2HPO_4/0.2M NaH_2PO_4, pH7.4, 100%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 temperature 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 δ 4.6 ~ 6.0 was excluded prior to principle component analysis (PCA) to remove the variations of water and urea. The data were normalized to the sum of the spectral integral to account for the differences between urinary concentrations.

Results and Discussion

Figure 1 shows the PCA results as score and loading plots for PC2 and PC8 which are the two largest components contributing to classification. Although all of the data points located dispersedly from each other due to intrinsic individual diversities in human, the subtle variations in metabolic profiling of these two groups are still detectable. The endogenous urinary metabolites responsible for the classification are listed in Table 1.

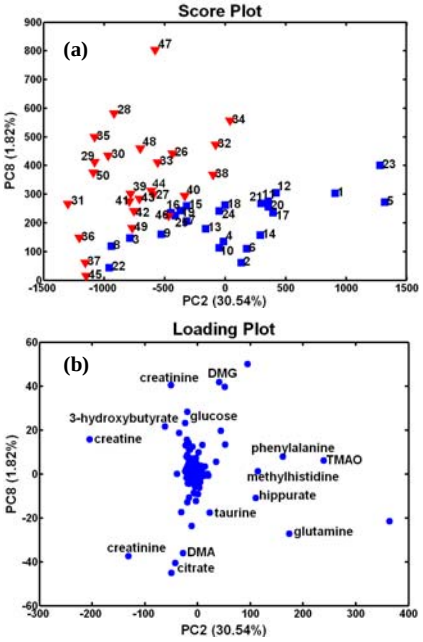

Fig.1 PCA results of control group (1-25, ■) and vegetarian group (26-50, ▼). (a) PC2 vs PC8 score plot, (b) PC2 vs PC8 loading plot.

A number of alterations were identified subject to long-term dietary differences. The most remarkable difference is the decreased level of creatinine, creatine, taurine, and trimethylamine-*N*-oxide (TMAO) which is correlated to deficiency of meat and fish intake in vegetarians [2]. Lower level of methylhistidine has been cited as a marker of deficient meat ingestion [3]. Citrate excretion linked with urinary pH value is increased by ingestion of vegetables and exclusion of animal protein and alcohol. Elevated hippurate is attributed to the microflora of gut in metabolizing plant phenolics and similar aromatic derivatives [4]. 3-Hydroxybutyrate, a kind of short chain fatty acids generated from microbial digestion of dietary fiber also increases. Glutamine is detected as a characteristic metabolite of the high-meat diet and urinary dimethylamine (DMA) is significantly associated with the consumption of fish. Besides, vegetarians have a lower level of glucose and insulin as well as a more favorable lipoprotein profile. The changes of dimethylglycine (DMG) and phenylalanine are also highlighted and further studies are in progress to identify the biomarkers of omnivorous and vegetarian diets.

Table 1. Endogenous urinary metabolites responsible for classification of control and vegetarian group

Metabolite	Chemical shift δ	Amount for vegetarian
3-Hydroxybutyrate	1.23	↑
Glutamine	2.08	↓
Citrate	2.57, 2.71	↑
DMA	2.73	↓
DMG	2.93	↓
Creatine	3.04, 3.93	↓↓
Creatinine	3.05, 4.08	↓↓
TMAO	3.27	↓↓
Taurine	3.42	↓↓
Glucose	3.69-3.81	↓
Hippurate	3.95,7.53	↑
Phenylalanine	7.42	↑
Methylhistidine	7.90	↓

Note: "↑" and "↓" denote higher or lower amount relative to the control group, and "↑↑" and "↓↓" denote much higher or much lower amount.

Conclusion

The effect of chronic dietary intervention on human urinary metabolic profiles was investigated in this study. A clear separation of omnivorous and vegetarian subjects was observed and the characteristic metabolites for low-meat plant-based diet were identified. These results are helpful for further studying the dietary and nutritional factors on human health as well as the dietary modulation during pathological state.

Acknowledgements

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