Susceptibility of Human Metabolic Phenotypes to Dietary Modulation

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Dietary composition has been shown to influence metabolism and to impact on the prevalence and risk for certain diseases, but hitherto, there have been no systematic studies on the effects of dietary modulation of human metabolic phenotype (metabotype). Here, we have applied 1H NMR spectroscopy in combination with multivariate statistical analysis to characterize the effects of three diets: “vegetarian”, “low meat”, and “high meat” on the metabotype signature of human participants. Twelve healthy male participants (age range of 25–74 years) consumed each of these diets, in a randomized order, for continuous 15-day-periods with an intervening washout period between each diet of 7 days duration. Each participant provided three consecutive 24-hour urine collections on days 13, 14, and 15 of each dietary period, and 1H NMR spectra were acquired on all samples. Pattern recognition analysis allowed differentiation of the characteristic metabolic signatures of the diets with creatine, carnitine, acetyl-carnitine, and trimethylamine-N-oxide (TMAO) being elevated in the high-meat consumption period. Application of orthogonal projection to latent structure discriminant analysis (O-PLS-DA) allowed the low-meat diet and vegetarian diet signatures to be characterized, and p-hydroxyphenylacetate (a microbial mammalian cometabolite) was higher in the vegetarian than meat diet samples, signaling an alteration of the bacterial composition or metabolism in response to diet. This work shows the potential for the routine use of metabonomics in nutritional and epidemiological studies, in characterizing and predicting the metabolic effects and the influence of diet on human metabotypes.

Keywords: metabonomics, protein, diet, vegetarian, human metabolism, NMR, pattern recognition, metabotype, phenotype

Introduction

A western lifestyle, characterized in part by a diet high in fat, red meat, and calories and low in fruits, vegetables, and fiber, has been associated with several chronic diseases including hypertension, diabetes, and coronary heart disease, as well as cancer.1–3 In particular, red meat has been associated with a variety of cancers, such as colorectal adenomas and carcinomas; furthermore, vegetarians have a lower level of glucose, insulin, and insulin resistance (IR), as well as a more favorable lipoprotein profile (increased HDL cholesterol levels and apoA1/apoB ratio in conjunction with reduced LDL levels).4–10

Although the long-term effects of different diets have been investigated, there is little understanding of the mechanisms of nutritional protection against disease. The evaluation of dietary intake is an essential part of many nutritional studies, and the most common methods of dietary assessment generally include quantitative food diaries and dietary recall or measurement of parameters such as blood glucose, IR, and so forth.11–13 However, these parameters do not tend to generate mechanistic information concerning dietary benefits, and dietary recall can be prone to lack of compliance.14,15 Recent proteomic studies have shown that short chain fatty acids (such as butyrate) generated from microbial digestion of dietary fiber may have important protective functions against gut cancers.16 Chung et al. have shown that N-butyrate specifically arrests the growth of colorectal cancer cells and induces apoptosis, suggesting new butyrate-induced protein-mediated mechanisms for these processes.
In this study, we have evaluated the variation in metabolic phenotype (metabotype) of human participants following different diets over time using high-resolution $^1$H NMR spectroscopy to generate information on the subtle diet-induced changes in the metabotype. The metabotype is defined as a “probabilistic multiparametric description of an organism in a given physiological state based on analysis of its cell types, biofluids or tissues”.

Any alteration of the physiological status can disrupt homeostasis, resulting in perturbations of the levels of endogenous biochemicals that are involved in key metabolic processes in the cells and tissues of an organism. To maintain homeostasis, and to adjust for the changes in tissue biochemistry, the compositions of body fluids are altered accordingly and lead to different and dynamic “metabotypes”, that may not be observable from gross examination of the phenotype. Thus, monitoring perturbations in biofluid composition may yield valuable information regarding underlying molecular mechanisms. The quantitative measurement of the dynamic multiparametric metabolic response of living systems to physiological stimuli or genetic modification is termed metabonomics, and largely relies on chemometric analysis of high-resolution spectral data. The use of $^1$H NMR spectroscopy to study the low MW (molecular weight) composition and molecular interactions in biological fluids, cells, and tissue extracts is well-established. $^1$H NMR spectra of biological materials can be collected quickly and efficiently, are rich in molecular structural and latent biological information, and allow a large range of low MW metabolites to be viewed simultaneously as a ‘metabolic fingerprint’. These properties make NMR uniquely suited for the noninvasive study of human populations. To simplify NMR data with a view to facilitating interpretation, automated data reduction followed by chemometric analysis has been extensively applied to NMR spectral data sets. Physiological variation per se can be of interest, particularly when considering health and lifestyle issues. However, the analysis of data from human studies is challenging due to the genetic and environmental diversity of human populations and the fact that protocols are generally not as tightly regulated as those for traditional toxicology screening in experimental animal models. To identify meaningful patterns in data where the primary aim is to characterize the functional consequences of disease or drug toxicity, elucidation of the effects of nutrition on the urinary biochemical profile must be established. Previous $^1$H NMR metabonomic investigations on human urine samples obtained under differing physiological states showed partial classification on the basis of participant, thereby highlighting inter-individual variability. In this study, chemometric methodologies, including PCA (principal component analysis) and O-PLS (orthogonal projection on latent structure) analysis, have been applied to the classification of urine samples obtained from healthy human individuals in relation to their dietary intake, using samples obtained from a controlled ‘metabolic suite’ study. In this metabolic study, healthy participants consumed a low-meat diet, a diet rich in red meat protein, and a diet with equivalent protein levels to the high red meat diet but from nonmeat sources in three randomly assigned 15-day dietary periods.

**Experimental Section**

**Study Protocol.** Twelve healthy, Caucasian, nonsmoking men, of normal body weight range (60–91 kg, mean of 76 kg) aged 25–74 were enrolled in this study. The protocol was approved by the Cambridge Local Ethics Committee. Studies with volunteers were carried out in a metabolic suite in two groups of six, where all food was provided and carefully controlled, and where all specimens could be collected and processed immediately. All food items were accurately weighed to the nearest gram, and main courses were prepared in advance and deep-frozen until required. Deionized water was used throughout for cooking and drinking. Three diets (i) low-meat diet (60 g/day), (ii) high red meat diet (420 g/day), and (iii) vegetarian diet (420 g/day from nonmeat sources) were randomly assigned in a crossover design in order to avoid an order effect. The 60-g meat diet contained 65-g/day of protein, and the high-meat and vegetarian diets contained 143–150-g/day of protein. Each dietary period lasted 15 days, and three 24-h urine collections were made after the volunteers had consumed each diet for 10 days. The participants acted as their own control, and the diets were isocaloric and kept constant in fat (30% total energy) by exchanging protein for carbohydrate. Basal diets contained 10 MJ per day, and individual energy requirements (to maintain constant body weight) were achieved using 1 MJ increments of bread, butter, and marmalade. A 3 day rotating menu was used, but the items were similar throughout to minimize day-to-day variation. Breakfast was composed of 40 g of cereal (cornflakes on the high-meat diet and Weetabix on the vegetarian diet), 120 g of bread (white bread on the high-meat diet and wholemeal bread on the vegetarian diet), 20 g of butter, 40 g of marmalade, and a daily allowance of 300 g of semi-skimmed milk. Lunch comprised of 100 g of roast beef given as a sandwich (120 g of white bread with 25 g of pickle, 20 g of butter, 50 g of cucumber, and 60 g of tomato), together with 150 g of apple. Wholemeal bread and egg or cheese (40 g) were substituted for the beef on the vegetarian diet. The red meat for the evening meal was cooked in sauces given in the form of 320 g (cooked weight) of minced beef pie, sweet and sour pork, and cottage pie. Vegetable and pasta bake, egg and chips, and vegetable and lentil bake were given for the vegetarian evening meals. Canned fruit (100 g) and ice cream were given for dessert. Volunteers were allowed to choose how much tea and coffee they consumed per day, but amounts were constant from day to day.

**Urinary Sample Preparation.** The 24 h urine collections (from 7 a.m. to 7 a.m.) were made into 2 containers containing 2 g of boric acid/L as a preservative. To monitor participant and dietary compliance, para- amino benzoic acid (PABA) tablets were given with each meal on the last 3 days of the dietary period, and the PABA-check method was employed to ensure a complete urine collection. Every volunteer provided three consecutive 24-h urine collections on days 13, 14, and 15 of each dietary period. To reduce the pH range of the samples, aliquots of urine samples (600 µL) were mixed with 300 µL of 100 mM phosphate buffer solution (made up in H$_2$O, pH 7.4). The samples were allowed to stand for 10 min prior to centrifugation at 8000 rpm for 5 min in order to remove insoluble material. Aliquots of the supernatants (600 µL) were mixed with 50 µL of TSP/D$_2$O solution (3-trimethylsilyl[2,2,3,3-$^2$H$_4$] propionate in deuterium oxide, 1 mM final concentration). TSP was added to act an internal chemical shift reference (δ$^1$H 0.0), and D$_2$O was added to provide a lock signal for the NMR spectrometer. Order effects in the statistical analysis were avoided due to the randomized crossover design used.

**600 MHz $^1$H NMR Spectroscopic Analysis of Urine Samples.** $^1$H NMR measurements were made on a Bruker AMX600 spectrometer operating at 600.13 MHz $^1$H frequency at ambient
is a short delay of typically 1.72 s. An additional relaxation delay (RD) of 2.0 s was added between pulses to allow TI relaxation. 1H NMR spectra were acquired with suppression of the water resonance using a standard presaturation pulse sequence comprising (RD-90°-τs-90°-τm-90°-acquire FID) (Bruker Biospin Germany) where τs is a short delay of typically 3 μs and τm is a mixing time (150 ms). Irradiation of the water signal was achieved during τm and RD. The summed FIDs were multiplied by an exponential weighting function corresponding to a line broadening of 0.3 Hz prior to Fourier transformation.

Preprocessing and 1H NMR Urine Spectra. To use as much relevant data as possible, full resolution 1H NMR spectra in the chemical shift range δ 0.0–10.0 were used for pattern recognition. The effect of variations in the presaturation of the water signal was removed by zeroing the intensity values in the region δ 4.5–4.9. All spectra were phase-corrected and referenced to the TSP resonance set at δ 0.0. A baseline correction was also applied to each spectrum using a polynomial curve fit, carried out using in-house software (Matlab Version 7.0, The Mathworks, MA) after importation of 42 000 points/spectrum, using spline cubic interpolation in order to give to all the spectra the same chemical shift scale. Finally, to remove concentration differences between dilute and concentrated urine samples, using full resolution data sets, all spectra were normalized to unit total integral. For some analyses, the aromatic region of the NMR spectra was omitted in order to investigate whether the presence of peaks from PABA metabolites interfered in the sample clustering in the PCA analysis.

Chemometric Analysis. 1. Principal Component Analysis (PCA). PCA is a projection method used for overviewing and explaining 'clustering' and trends within multivariate data and to detect outlier samples. The data are reduced to a few latent variables (or principal components) describing maximum variation within the data. The principal components (PCs) consist of a set of scores, which highlight clustering, trends, or outliers, in the observation (or sample) direction in the data matrix. A corresponding set of 'loadings' focuses on the variation in the variable direction, describing the influence from the variables on the scores. The nonsystematic part of variation not explained by the models forms the residuals.

2. O-PLS (Orthogonal Projection to Latent Structures) Discriminant Analysis. While PCA explains the maximum variance in the data set, PLS defines the maximum separation between defined class samples in the data, and for this reason, class membership of each sample must be known. Once a PLS model is calculated and validated, it can be used for prediction of class membership for unknown samples. O-PLS is an extension of the PLS chemometric method for multivariate data that models sources of variation from X (NMR spectra) that is not related to Y (class, i.e., dietary regimen). One special case of PLS is O-PLS-Discriminant Analysis (O-PLS-DA) where PLS is used to find the relationship between the descriptors in X and the class identity of the samples, described by a dummy matrix containing the class information. These sources of variation may disturb the multivariate modeling, cause imprecise predictions for new samples, and also affect the robustness of the method over time. For the removal of such undesirable systematic variation in the data, different types of preprocessing methods prior to PCA or PLS have been commonly used, such as Orthogonal Signal Correction (OSC). However, the advantage of O-PLS compared to OSC is that the orthogonal variation partitioning is implemented in the algorithm and avoids the two step process, OSC followed by PLS, making the validation of the model easier and more robust. The interpretation is also improved because any "structured noise" is modeled separately from the data variation common to X (the NMR spectra) and Y (the class variable).

The method used in this study to interpret the O-PLS coefficients (or loadings relating to the relative influence of a particular signal or metabolite on class discrimination) is to calculate a model based on unit variance scaled data, and then, in a first step, back-transform the predictive O-PLS coefficients by multiplying all values by their respective standard deviation. In a second step, the back-transformed loading is plotted using a color corresponding to the coefficient value in the model that represents the correlation of the variable X (NMR data) with Y (class or dietary intervention in this case) for each data point. The scale range of colors is set using the maximum and the minimum of the unit variance scaled model weight. The interpretation of the loadings is therefore straightforward for the spectroscopist because the resulting plot provides a loading with the same shape as that of a spectrum (based on the covariance matrix), but on the same plot, the important variables for the discrimination between the classes (calculated from the correlation matrix) are highlighted by the color code.

Results and Discussion

Typical examples of 1H NMR spectra of urine samples obtained from a participant representing each of the dietary regimes are depicted in Figure 1. Resonance assignments were performed according to the existing literature. The most marked differences, observable on the 1H NMR spectra between the three diets, included elevated urinary levels of creatinine, taurine, carnitine, trimethylamine-N-oxide (TMAO), and methylnitrosamine. These perturbations were consistent among the three samples for each participant and relate to the high-meat group. The spectral changes differentiating low-meat and vegetarian groups were, on the other hand, more subtle. The split observed in the NMR signal of citrate (Figure 1) is due to complexation with boric acid. The effects of adding boric acid to biological samples as a preservative has been studied previously and found to have little or no influence on the pattern recognition analysis.

PABA has long been used as a marker of completeness of 24 h urine samples in nutritional epidemiological studies and prescribed diet regimes. The conventional PABA recovery cutoff for completeness is 85% or above. Colorimetric or HPLC methods are used for measuring PABA and its conjugated metabolites in human urine after oral administration. In the current study, dietary compliance was found to be acceptable for all participants on all diets.

The chemometric analysis has been performed both with and without inclusion of the aromatic region (δ 10–4.3) of NMR spectra in order to evaluate whether PABA metabolites interfere with the pattern recognition. Results obtained without the inclusion of the aromatic region were not different from those obtained with the full-spectrum range, indicating that the presence of PABA did not influence the pattern recognition (data not shown). Initial analysis of the data set was conducted with no knowledge as to the dietary status of the samples from each individual using PC analysis (with unit variance scaling) with and without taking account of the aromatic region. Inter-individual variation dominated the PCA analysis (Figure 2A).
with some participants showing a much greater metabolic variation than others. For example, samples from participant 1 show a tighter clustering than the samples from participant 6. However, intra-individual variation was dominated by dietary exposure, and analysis of samples from each participant revealed three clusters (Figure 2B) relating to each of the three diets.

With reference to the loadings (variable coefficients) for the spectral descriptors, it could easily be established which cluster related to the high-meat diet. The most marked differences relating to the high-meat diet included elevated urinary levels of creatinine, creatine, TMAO, taurine, and 1- and 3- methylhistidine. These perturbations were consistent among the samples identified as high-meat, and metabolites such as carnitine and 1- and 3-methylhistidine have been cited in the literature as markers of meat ingestion.42 In one epidemiological study, monitoring of urinary methylhistidine excretion as a marker of meat consumption was used to successfully predict vegetarian status in a study of 126 individuals based on food frequency questionnaires.43

For the two remaining diets (low-meat and vegetarian), the spectral changes between the two were more subtle, and this was confirmed with PCA analysis (data not shown): clear, but incomplete separation of the high-meat diet was observed, while the low-meat and vegetarian diet participants were partially comapping. O-PLS-DA analysis was applied to the data matrix to remove variation in \( X \) (NMR spectra) that was unrelated (orthogonal) to \( Y \) (diet). In this study, removal of nonrelated noise resulted in a much improved separation of the low-meat diet and the vegetarian diet, and all three dietary regimes formed distinct clusters as shown in Figure 3A. The application of O-PLS-DA produced a regression model (Figure 3B) describing the maximum separation between predefined classes, which focused on the actual class discriminating variation in the data. Since the principle aim of the study was to explore the metabolic consequences of a diet with high-meat protein content, analysis was focused on the differentiation between the high-meat and vegetarian diets which contained the same amount of protein (143–149 g protein/day). The O-PLS-DA loadings for the pairwise discrimination of the high-

Figure 1. \(^1\)H NMR spectra of urine from a single individual on each of the three diets (HM = high meat; LM = low meat, and VE = vegetarian): only significant metabolites have been labeled for each of the three urinary metabolic profiles.
meat and vegetarian groups are presented in Figures 4 and 5, respectively. The color scale corresponds to the UV model variable weights and allows the identification of significant class-discriminating metabolites (Table 1), responsible for the clustering patterns.

The O-PLS-DA coefficients of the high-meat group (Figure 4) show that the urinary concentration of creatine and creatinine (Figure 4) are much greater than those from the low-meat (figure not shown) and vegetarian groups (Figure 5). Creatine is a metabolite that is produced naturally by the human body, and it is found mainly in the red muscle tissue, but it is also present in other tissues with high and fluctuating energy demands such as brain, retina, and testes.44 Normally, creatine, which functions to increase the availability of cellular ATP (adenosine triphosphate), is acquired through regular dietary intake of products such as meat and fish, which are high in creatine/creatinine, so that urinary levels increase in response to meat consumption. Although creatinine excretion is related to body weight, creatinine excretion therefore is not constant as it varies from day to day according to the amount of meat consumed.45

The O-PLS-DA coefficients of the carnitine peak and acetylcarnitine indicate that the highest concentrations of these metabolites are found in the high-meat group (Figure 4 and Table 1), and these metabolites are thus responsible for the clustering separation of this group from the low-meat and vegetarian groups. Carnitine, a trimethylated amino acid, is a cofactor required for transformation of free long-chain fatty acids into acylcarnitines and for their subsequent transport into the mitochondrial matrix, where they undergo β-oxidation for cellular energy production. Mitochondrial fatty acid oxidation is the primary energy source in heart and skeletal muscle. It is assumed that a diet adequate in protein will supply enough exogenous, and promote any additional endogeneous, synthesis needed to supply an individual’s requirements for l-carnitine. However, since l-carnitine is found primarily in animal proteins, with red meat regarded as the richest source, it is theoretically possible to consume a high protein diet consisting of beans, legumes, or egg whites and still promote a relative deficiency. Since a vegetarian diet is very low in exogenous carnitine, and is potentially low in some of the substrates required for carnitine synthesis, there is some concern that following a strict vegetarian diet might produce a carnitine deficiency in some individuals.46 Since adequate dietary lysine is required as a substrate for carnitine synthesis, a deficiency of this amino acid or the other cofactors—iron, vitamin C, B6, niacin—might also compromise carnitine status.

The urinary concentration of trimethylamine-N-oxide (TMAO) and taurine after ingestion of a high-meat diet is higher in comparison to the low-meat and vegetarian diets (Figure 4 and Table 1). TMAO has been correlated to meat and fish ingestion, but the formation of TMAO and γ-butyrobetaine from l-carnitine by enteric bacteria has also been demonstrated in rats and humans.47 While TMA (trimethylamine) and TMAO are generally regarded as nontoxic substances, they are of clinical interest because of their potential to form the carcinogen N-nitrosodimethylamine.48 The excretion of the amino acid taurine, a conditionally essential amino acid playing an im-

![Figure 2](image-url)  
**Figure 2.** (A) PC analysis performed on all data and color-coded according to participant. (B) PC analysis performed on one participant and color-coded according to the diet.
important role in many physiological functions, is also increased following a high-meat diet (Figure 4 and Table 1). This metabolite is commonly associated with ingestion of a high amount of proteins (Atkins diet), and urinary taurine excretion in vegan subjects is about half the value of that in the omnivores.49,50

Figure 4. The loading coefficient plot of the high-meat group corresponding to the scores plot represented in Figure 3A: the signal orientation indicates the higher (positive orientation) or lower (negative orientation) concentration of characteristic metabolites. The color of the signal reflects the significance of the correlation with diet (red indicates highest significance and blue indicates no significance).

Figure 5. The loading coefficient plot of the vegetarian group corresponding to the scores plot represented in Figure 3A: the signal orientation indicates the higher (positive orientation) or lower (negative orientation) concentration of characteristic metabolites. The color of the signal reflects the significance of the correlation with diet (red indicates highest significance and blue indicates no significance). The region between δ 6.85 and 7.20 is expanded to show the other resonances of p-hydroxyphenylacetate.
A high concentration of N-acetyl-5-hydroxytryptamine was found in the high-meat urinary profile. This compound, also known as N-acetylserotonin, is produced in one of the steps of tryptophan metabolism (tryptophan → 5-hydroxytryptophan → 5-hydroxytryptamine → N-acetyl-5-hydroxytryptamine). The amount of tryptophan (an amino acid necessary for normal growth in infants and for nitrogen balance in adults) in the foods that are eaten has only a small influence upon the amount of tryptophan that enters the brain. Large-molecule amino acids, among them tryptophan, compete with each other to enter “gates” between the circulating blood stream and the relatively confined brain fluids.\(^5\) A high-protein meal (e.g., high in meat, dairy products, and eggs) provides many other amino acids that compete with tryptophan for entry into the brain; the end result is less tryptophan passing into the brain, a decrease in the synthesis of serotonin in the brain, which may explain the high amount of N-acetyl-5-hydroxytryptamine in the urine.\(^5\) Conversely, a low-protein, carbohydrate-rich diet (full of starches, vegetables, and fruits) results in the highest levels of serotonin in the brain, because fewer large-molecule amino acids are competing with tryptophan to enter the brain.\(^5\) Another characteristic metabolite of the high-meat group is glutamine, which is not an essential amino acid, since it can be readily produced from glutamate in all tissues, with muscle tissue being the primary source of glutamine in the blood. Although there is no dietary reference intake (for under normal conditions, glutamine is not a necessary dietary constituent), during critical illness, severe trauma, intestinal disease, starvation, total parenteral nutrition (intravenous feeding), wasting (excess loss of lean body mass), and extreme endurance exercise, the body’s need and consumption of glutamine can exceed the ability of tissues to produce this amino acid. Under such stress conditions, dietary glutamine is beneficial. However, while glutamine is nontoxic and probably harmless, the benefits of consuming dietary supplements containing this amino acid have not been proven.\(^5\) Low glutamine levels in plasma may reflect chronic insufficient essential amino acid dietary intake, and this may lead to ammonia detoxification problems.\(^5\)

Characteristic NMR peaks found for the urines of the vegetarian group can be assigned to p-hydroxyphenylacetate (Figure 5). Tyrosine from dietary protein is the parent compound from which p-cresol, p-hydroxybenzoate, and p-hydroxyphenylacetate are formed (Figure 6A). These compounds are not products of normal human metabolism but are produced by bacteria and protozoa that populate the gut.\(^5\) Both the transamination of tyrosine to form p-hydroxyphenylacetate (HPA) and the decarboxylation to p-cresol are carried out by a limited number of microfloral species including

![Figure 6](image)

**Figure 6.** (A) Tyrosine metabolism; (B) L-carnitine biosynthesis.

*Clostridium difficile.* Since *Proteus vulgaris* can do only the first of these steps, HPA will increase in urine if *P. vulgaris* is the predominant organism. This might indicate that some compounds that appear in urine, such as p-hydroxyphenylacetate, are unique metabolic products of the microbes that inhabit the lumen of gut, which is strictly dependent on the dietary regime.\(^5\) In addition, p-hydroxyphenylacetic aciduria has been found useful in detecting small bowel disease associated with anaerobic bacterial overgrowth.\(^5\) Another compound characteristic of the urinary profile of the vegetarian group is N\(^{6}\)-N\(^{6}\)-N\(^{6}\)-trimethyllysine (TML): the negative loading coefficient indicates a low amount of this metabolite at urinary level following a vegetarian diet. TML is generated within lysosomes by the hydrolysis of proteins containing lysines that are trimethylated at their \(\epsilon\)-amino group by a protein-dependent methyltransferase (Figure 6B). Apart from the dietary intake of carnitine, most eukaryotes are able to synthesize this compound from TML, and this would explain the low levels of TML following a vegetarian diet.

### Table 1. List of Most Characteristic Metabolites Found in the High-Meat and Vegetarian Groups Obtained with O-PLS-DA Analysis

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Chemical Shift</th>
<th>HM Correlation</th>
<th>HM Covariance</th>
<th>LM Correlation</th>
<th>LM Covariance</th>
<th>VE Correlation</th>
<th>VE Covariance</th>
</tr>
</thead>
<tbody>
<tr>
<td>creatine</td>
<td>3.934 (s)</td>
<td>0.75</td>
<td>-0.38</td>
<td>-0.38</td>
<td>0.45</td>
<td>-0.23</td>
<td>-0.22</td>
</tr>
<tr>
<td>taurine</td>
<td>3.425 (s)</td>
<td>0.66</td>
<td>-0.32</td>
<td>-0.35</td>
<td>0.05</td>
<td>-0.02</td>
<td>-0.02</td>
</tr>
<tr>
<td>TMAO</td>
<td>3.269 (s)</td>
<td>0.71</td>
<td>-0.47</td>
<td>-0.23</td>
<td>0.71</td>
<td>-0.47</td>
<td>-0.23</td>
</tr>
<tr>
<td>carnitine</td>
<td>3.228 (s)</td>
<td>0.81</td>
<td>-0.49</td>
<td>-0.33</td>
<td>0.17</td>
<td>-0.10</td>
<td>-0.07</td>
</tr>
<tr>
<td>acetyl carnitine</td>
<td>3.195 (s)</td>
<td>0.70</td>
<td>-0.42</td>
<td>-0.28</td>
<td>0.03</td>
<td>-0.02</td>
<td>-0.01</td>
</tr>
<tr>
<td>creatine</td>
<td>3.041 (s)</td>
<td>0.72</td>
<td>-0.33</td>
<td>-0.38</td>
<td>0.49</td>
<td>-0.23</td>
<td>-0.26</td>
</tr>
<tr>
<td>glutamine</td>
<td>2.445 (m)</td>
<td>0.72</td>
<td>-0.32</td>
<td>-0.40</td>
<td>0.01</td>
<td>-0.01</td>
<td>-0.01</td>
</tr>
<tr>
<td>p-hydroxyphenylacetate(^6)</td>
<td>3.452 (s)</td>
<td>-0.44</td>
<td>-0.24</td>
<td>0.69</td>
<td>-0.04</td>
<td>-0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>N-acetylglutamate</td>
<td>2.035 (s)</td>
<td>-0.49</td>
<td>-0.31</td>
<td>0.82</td>
<td>-0.03</td>
<td>-0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>(N,N,N)-trimethyllysine</td>
<td>3.112 (s)</td>
<td>-0.11</td>
<td>0.57</td>
<td>-0.47</td>
<td>0.02</td>
<td>0.08</td>
<td>-0.07</td>
</tr>
</tbody>
</table>

\(^a\) Doublet at 6.867 and doublet at 7.172.
Conclusions

A clear separation of the urinary metabolic profiles according to the type of diet and the use of O-PLS-DA analysis allowed the identification of the characteristic metabolites for each diet and nutrition in the development of human diseases. Metabotype which could provide valuable information in genetic and environmental factors, with diet representing a potential target for intervention. Some of these studies, where it is useful to be able to distinguish the perturbations in specific metabolic pathways and disease risk or prevalence may be explored.

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