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Analytical Biochemistry 352 (2006) 274–281
ANALYTICAL BIOCHEMISTRY
www.elsevier.com/locate/yabio

Probing gender-specific metabolism differences in humans by nuclear magnetic resonance-based metabonomics

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Received 23 December 2005
Available online 20 March 2006

Abstract

The measurement of metabolite profiles that are interpreted to yield biomarkers using multivariate data analysis is now a well-established approach for gaining an improved understanding of the impact of genetic modifications, toxicological and therapeutic interventions, and exposure to stimuli (e.g., noxious agents, stressors, nutrients) on the network of transcripts, proteins, and metabolites present in cells, tissues, or whole organisms. This has been termed *metabonomics*. In this study, multivariate analysis of ^{1}H nuclear magnetic resonance (NMR) spectra of metabolite profiles of urine and plasma from 150 healthy humans revealed that in young people and/or individuals with low body mass indexes, females had higher rates of lipid biosynthesis than did males, whereas males had higher rates of protein turnover than did females. With increasing age, overall lipid biosynthesis decreased in females, whereas metabolism increasingly favored lipid synthesis over protein turnover in males. By relating the derived metabonomic data to known metabolic pathways and published biochemical data, it appears that females synthesize relatively more lipoproteins and unsaturated lipids than do males. Furthermore, the changes in lipid biosynthesis and urinary citrate excretion in females showed a positive correlation. Estrogen most likely plays an essential role in the regulation of, and communication between, protein and lipid biosynthesis by controlling pH in mitochondria and the cytoplasm and hence the observed altered citrate levels.

Keywords: Metabonomics; Chemometrics; NMR spectroscopy; Gender variation; Citric acid cycle

Because of the need to understand the biochemical basis of a healthy individual, biomedical research must face the challenge of elucidating the relationship between health, disease, and metabolism and the effects of nutrition, pharmaceuticals, and environmental factors. Global profiling tools are required to fully understand the impact of genetic modifications and toxicological interventions and exposure to stimuli (e.g., noxious agents, stressors, nutrients, genetic modification, pathophysiological or environmental conditions) on the network of transcripts, proteins, and metabolites found within cells, tissues, or organisms. These new "-omics" sciences, comprising gene expression (transcriptomics), protein profiling (proteomics), and metabolite profiling (metabonomics), have become key technologies for measuring and describing altered biological function at several levels of biomolecular organization [1,2]. In particular, metabonomics characterizes the ways in which the relative concentrations of endogenous small molecule components in biofluids and tissues of complex organisms vary over time as the result of some stimulus [3,4]. The primary goals of metabonomics are to identify metabolic biomarkers or predictors associated with a specific biochemical event and to relate these to the mechanism of effect.

High-resolution ^{1}H nuclear magnetic resonance (NMR)[1] spectroscopy is an efficient and nondestructive tool for

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[1] *Abbreviations used:* NMR, nuclear magnetic resonance; BMI, body mass index; TSP, 3-(trimethylsilyl)-[2,2,3,3-$^{2}H_4$]-1-propionate; TBI, triple resonance broadband inverse; FID, free induction decay; PCA, principal components analysis; PLS-DA, partial least-squares discriminant analysis; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; TMAO, trimethylamine-*N*-oxide; CoA, coenzyme A.

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doi:10.1016/j.ab.2006.02.033

generating data on a multitude of metabolites in biofluids or tissues [5,6]. The acquired spectral profile of a biofluid, such as urine, plasma, or saliva, reflects the metabolic status of the organism, which changes in response to stressors so as to maintain a homeostatic balance [7]. Information recovery, in terms of relationships between the NMR spectral profiles and their biochemical interpretation, can be maximized by applying multivariate statistical tools [8] to analyze the information-rich NMR data. ^{1}H NMR spectroscopy of complex biological mixtures coupled with multivariate statistical analysis allows better visualization of the changing endogenous biological profile in response to physiological challenge or stimulus such as the disease process, administration of a xenobiotic [9–11], environmental stress [12], genetic modification [13,14], changes in nutrition [15–17], and other physiological effects [18].

As a first step toward understanding nutritional or environmental effects on metabolic changes, plasma and urine NMR profiles have been acquired on a healthy human control population. Interpretation of the data in this lifestyle database provides information on the metabolite compositions of each biological fluid, the interindividual profile variability, and the impact of key parameters such as gender, age, body mass index (BMI), and lifestyle.

Materials and methods

Human participants

Healthy adult human volunteers (84 women and 66 men, hereafter referred to as females and males, respectively) were recruited at the Nestlé Research Center in Lausanne, Switzerland, during May and June 2003. All volunteers completed confidential health and lifestyle (e.g., sport/exercise activities, alcohol and coffee consumption, specific dietary regimes) questionnaires, reported gender and age, and signed informed consent forms to be participants in the study. Acutely ill individuals (those suffering from pyrexia, common cold, or influenza and those taking antibiotic or antiinflammatory therapy) and pregnant females were excluded from the study. Urine and blood samples were collected from females from the 10th to 15th days of the menstruation cycle. Demographic and lifestyle data, coded to protect confidentiality, were entered into an electronic patient diary (CRF, Helsinki, Finland) as per good clinical practice.

Blood and urine samples

Blood (2 ml) from fasted participants was drawn from the antecubital vein into heparinized Sarstedt syringes (Nümbrecht, Germany). Plasma was immediately separated by centrifugation at 8000*g* for 10 min at 4 °C and was stored at −80 °C for subsequent metabolic profiling. For NMR analysis, the plasma samples were diluted with 2 volumes of phosphate buffer (0.2 M Na_2HPO_4/0.2 M NaH_2PO_4 in 80% H_2O/20% ^2H_2O (p^2H 6.0), 5 mM imidazole, 3 mM sodium azide, and 1 mM sodium 3-(trimethylsilyl)-[2,2,3,3-$^2H_4$]-1-propionate (TSP) (NMR chemical shift reference $\delta = 0$)) and centrifuged at 10,000*g* for 5 min at 4 °C prior to analysis.

A minimum of 20 ml urine was collected by each participant from the second morning urine. Samples were frozen after being delivered to the Metabolic Unit and were stored at −20 °C. For NMR analysis, a 1-ml sample of urine was lyophilized and subsequently reconstituted to 700 µl with deuterated phosphate buffer (0.2 M Na_2HPO_4/0.2 M NaH_2PO_4 in ^2H_2O (p^2H 6.0), 0.5 mM TSP, 5 mM imidazole, and 3 mM sodium azide (bactericide)). The samples were centrifuged at 1600*g* for 5 min at 10 °C prior to NMR analysis.

NMR spectroscopy

One-dimensional ^{1}H NMR spectra were acquired on a Bruker DRX-600 NMR spectrometer operating at a proton NMR frequency of 600.13 MHz. Samples were measured in 5-mm NMR tubes at a temperature of 27 °C. A triple resonance broadband inverse (TBI) probe and a sample changer for sample delivery were used. For all samples, a standard one-dimensional ^{1}H pulse sequence with water presaturation (NOESYPRESAT) was applied. In addition, spin-echo spectra using the Carr–Purcell–Meiboom–Gill sequence ($D[-90°-(\tau-180°-\tau)_n$–FID]) were recorded for plasma samples. This pulse sequence attenuates the NMR signals from slowly tumbling molecules such as proteins and retains those of low-molecular weight compounds and some lipid components. The spin-echo loop time ($2n\tau$) was set at 64 ms. A total of 256 and 128 transients were collected for urine and plasma, respectively. Typical acquisition parameters included 32,000 data points, a spectral width of 8389 Hz, an acquisition time of 1.95 s, and a relaxation delay (D) of 2 s. As with the standard one-dimensional spectra, an exponential line-broadening function of 0.3 Hz was applied to the free induction decay (FID) prior to Fourier transformation. All spectra were phase and baseline corrected manually. The urine spectra were referenced to TSP (δ 0), and the plasma spectra were referenced to the methyl peak of lactate (δ 1.34). All recorded profiles were visually inspected for acceptability and were analyzed by multivariate statistical methods.

Multivariate statistical analysis

Multivariate analyses, such as principal components analysis (PCA) and partial least squares discriminant analysis (PLS-DA), were performed using SIMCA-P+ (version 11, Umetrics, Sweden) and MatLab (MathWorks, Natick, MA, USA), including the PLS Toolbox 3.0 (Eigenvector Research, Manson, WA, USA). Using an in-house routine written in MatLab, each NMR spectrum was reduced to a smaller number of segments of width 0.02 ppm and the total signal integral within each was integrated. In regions where large variations in chemical shift due to variations in pH were expected, wider segment widths were used.