Analytica Chimica Acta 908 (2016) 121–131

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Postprandial metabolomics: A pilot mass spectrometry and NMR study of the human plasma metabolome in response to a challenge meal

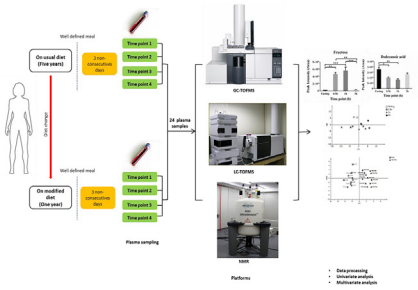
Masoumeh Karimpour [a], Izabella Surowiec [a], Junfang Wu [a], Sandra Gouveia-Figueira [a, b], Rui Pinto [a, c], Johan Trygg [a], Angela M. Zivkovic [d], Malin L. Nording [a, *]

[a] Computational Life Science Cluster (CLiC), Department of Chemistry, Umeå University, 90187 Umeå, Sweden
[b] Department of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden
[c] Bioinformatics Infrastructure for Life Sciences, Sweden
[d] Department of Nutrition, University of California, Davis, One Shields Ave, CA 95616, USA

HIGHLIGHTS

- GC-MS, LC-MS and NMR platforms to cover a wide range of metabolites in plasma.
- Univariate and multivariate data analysis.
- Stable postprandial response over time largely independent of the background diet.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 July 2015
Received in revised form
25 November 2015
Accepted 1 December 2015
Available online 17 December 2015

Keywords:
Metabolomics
Postprandial
Multi-platform analysis

ABSTRACT

The study of postprandial metabolism is relevant for understanding metabolic diseases and characterizing personal responses to diet. We combined three analytical platforms – gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) – to validate a multi-platform approach for characterizing individual variation in the postprandial state. We analyzed the postprandial plasma metabolome by introducing, at three occasions, meal challenges on a usual diet, and 1.5 years later, on a modified background diet. The postprandial response was stable over time and largely independent of the background diet as revealed by all three analytical platforms. Coverage of the metabolome between NMR and GC-MS included more polar metabolites detectable only by NMR and more hydrophobic compounds detected by GC-MS. The variability across three separate testing occasions among the identified metabolites was in the range of 1.1–86% for

Abbreviations: ANOVA, ANalysis Of VAriance; CV-ANOVA, Cross Validation-ANalysis Of VAriance; MFE, Mass Feature Extraction; MVA, multivariate analysis; OPLS, orthogonal projections to latent structures; OPLS-DA, orthogonal projections to latent structures-discriminant analysis; PCA, principal component analysis; SMC, Swedish Metabolomics Centre; SUS, shared and unique structure; UPSC, Umeå Plant Science Centre; CV, coefficient of variation; EDTA, ethylene diamine tetra-acetic acid; GC-MS, gas chromatography-mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; NMR, nucleic magnetic resonance.

* Corresponding author.
E-mail address: malin.nording@umu.se (M.L. Nording).

http://dx.doi.org/10.1016/j.aca.2015.12.009

Data analysis
Mass spectrometry
Nucleic magnetic resonance

GC-MS and 0.9–42% for NMR in the fasting state at baseline. For the LC-MS analysis, the coefficients of variation of the detected compounds in the fasting state at baseline were in the range of 2–97% for the positive and 4–69% for the negative mode. Multivariate analysis (MVA) of metabolites detected with GC-MS revealed that for both background diets, levels of postprandial amino acids and sugars increased whereas those of fatty acids decreased at 0.5 h after the meal was consumed, reflecting the expected response to the challenge meal. MVA of NMR data revealed increasing postprandial levels of amino acids and other organic acids together with decreasing levels of acetoacetate and 3-hydroxybutanoic acid, also independent of the background diet. Together these data show that the postprandial response to the same challenge meal was stable even though it was tested 1.5 years apart, and that it was largely independent of background diet. This work demonstrates the efficacy of a multi-platform metabolomics approach followed by multivariate and univariate data analysis for a broad-scale screen of the individual metabolome, particularly for studies using repeated measures to determine dietary response phenotype.

1. Introduction

Postprandial metabolism displays dynamic features after meal ingestion [1,2]. It depends on a variety of factors including meal composition, gender, and age and may be measured with a wide range of endpoints [3–6]. Metabolomics has been used to assess individual metabolic responsiveness to a lipid challenge [7], altered metabolic pathways in diabetes [8], and postprandial insulin demand [9], just to mention a few examples. The common goal has been to identify quantitative and dynamic indicators of an individual's response to a meal challenge, thereby disclosing subtle effects of diet on human metabolic status. This was emphasized in the Pellis et al. [10] study, which investigated the effects of a dietary intervention by measuring both the fasting baseline as well as the postprandial metabolic response. The authors found that a standardized perturbation of metabolic homeostasis induced by a meal challenge was more informative of dietary effects than a quantification of the homeostatic (fasting) state alone. As such, postprandial metabolomics holds the promise of providing a comprehensive health assessment in relation to different dietary interventions for the purpose of preventing disease and optimizing health at a personalized level.

The most widely employed techniques in metabolomics are based on nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) [11,12]. Because of the ease of sample preparation, high throughput, and high reproducibility, NMR based metabolomics has been widely used in many fields, for instance in drug toxicity studies [13], environmental monitoring or assessment [14], and pharmacological drug discovery [13–16]. NMR has also proven to be an effective tool in dietary research [10] and in studies of diseases that can be at least partly related to dietary regime, such as high blood pressure and diabetes [17]. Metabolomics studies using MS-based platforms preceded by gas chromatography (GC) or liquid chromatography (LC) separation have also been extensively undertaken [18]. Recently, GC-MS and LC-MS approaches have been frequently used for metabolic profiling of plasma and serum [19,20] in diet related research [21]. LC-MS has several advantages over GC-MS metabolic profiling. For example, there is no need for sample derivatization, and more polar and higher molecular weight molecules may be analyzed. On the other hand, compound deconvolution and especially compound identification are more challenging in LC-MS. This is because GC-MS libraries are standardized and comparable between different instruments. Also, retention indices can be readily compared between laboratories. This is not the case for LC, where retention times are more column and instrument dependent and therefore have to be obtained on the same instrument. Furthermore, MS/MS spectra used for compound identification in LC-MS analysis are less repeatable between instruments from different vendors than the electron impact fragmentation spectra used in GC-MS. This makes reliable identification of LC-MS peaks strongly dependent on the existence of extensive in-house mass spectra libraries.

No current analytical platform is able to detect all metabolites in plasma or any other biological matrix. However, several platforms may complement each other to give a more comprehensive coverage of metabolites. Application of platforms exploiting different separation (LC and GC) and detection techniques (NMR and MS) facilitates a broad scale characterization of metabolite content. A certain degree of overlap is unavoidable, as shown by Tredwell et al. [11]. However, the extent of overlapping vs unique features extracted from the postprandial human plasma metabolome by GC-MS, LC-MS and NMR are largely unknown. Moreover, the overlapping or shared metabolites can be used to cross-validate the results of multiple platforms and ensure high data quality. Here we used all three analytical platforms to measure the postprandial plasma metabolome of one individual to demonstrate the feasibility of these platforms for measuring postprandial metabolism. We measured the postprandial response of the individual on the same meal three times on a usual background diet and three times on a modified background diet to explore whether diet-specific responsiveness in certain metabolites may be detected and to determine which analytical platform is most suited for analysis of personalized postprandial metabolomic profiles, or whether a combination of multiple platforms is the best choice.

2. Materials and methods

2.1. Chemicals

Detailed information of chemicals used for all three platforms is given in the Supporting Information.

2.2. Study design

The study protocol followed the principles on human research ethics in the Declaration of Helsinki and was approved by the Institutional Review Board of the University of California, Davis. The subject was recruited in response to a flyer on the University of California Davis campus. The subject agreed to participate in the study after it was determined that she met eligibility criteria and after formal consent was obtained. Inclusion criteria included the ability to donate blood safely before and three times after consuming a challenge meal of her choice. The subject ate the food of her choice at all times and changed her diet from vegan to vegetarian (hereafter called the usual and modified background diets respectively) of her own free will. The subject was tested on