Nutrimetabolomics[1,2]

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ABSTRACT

Metabolic pathways are tightly regulated in a tissue-specific manner to maintain whole-body homeostasis. Nutrients and hormones control these pathways at the level of transcription, translation, and/or post-translation. Genomic and proteomic tools have been predominantly used to understand metabolic regulation, and only a few studies used metabolomics approaches. Metabolomics is a powerful, unbiased approach that allows comprehensive metabolic analysis of physiologic measurements and energy balance. Thus, nutrimetabolomics can expedite our ability to identify metabolic diseases that are influenced by nutrients and to develop targeted diet-based treatments. Presentations at this symposium reviewed current resources and platforms for metabolic profiling along with statistical and bioinformatics tools for data and pathway analyses. Specific applications of metabolomics were illustrated in nutritional and disease conditions, including polycystic ovary syndrome, diabetes, and obesity and in host-gut microbiome interactions. *Adv Nutr 2014;5:792–794.*

Introduction

Dietary factors alter metabolism via numerous and diverse mechanisms that tightly control tissue-specific responses as well as whole-body metabolism and homeostasis. This regulation often occurs through changes in the transcriptome, proteome, and metabolome. Until recently, these "omics" approaches were heavily focused on proteomic and genomic tools. In contrast, very few studies applied metabolomics tools to profile changes in the whole metabolome in health or disease states. Metabolomics is a powerful, unbiased approach that allows comprehensive metabolic analysis of physiologic measurements and energy balance. Thus, unbiased systems-based nutrimetabolomics studies can expedite our ability to identify metabolic diseases that are influenced by nutrients and to develop personalized diet-based treatments.

This symposium provided an overview of current state-of-the-art metabolomics technologies applied to cells, animals, or human subjects in nutritional and disease conditions. Relevant resources and challenges related to statistical and bioinformatics analyses were discussed.

Dr. Shankar Subramanian Provided an Overview of Metabolomics, Systems Biology, and Bioinformatics

Dr. Shankar Subramanian is a Professor of Bioengineering, Chemistry, and Biochemistry, Cellular and Molecular Medicine, and Nano Engineering. He is currently the Joan and Irwin Jacobs Endowed Chair in Bioengineering and Systems Biology at the University of California at San Diego.

Dr. Subramanian gave a review of metabolomics and its importance as the functional endpoint of physiologic pathways and mechanisms. One key benefit is that metabolomics can be conducted in specimens of different sources, such as serum and tissue. It also allows researchers to analyze the direct input of environmental influences. In addition, newer technology is making it increasingly possible to conduct quantitative individual metabolomics analyses.

Metabolomics applications have been limited, in part, due to challenges that include the following: issues with the dynamic range of metabolites, identifying how to quantitate metabolites, and finding user-friendly methods to integrate the metabolomics data with other "omics" data. This is an area in which Dr. Subramanian's team has contributed substantially. They recently launched a metabolomics analysis Web site (1) to allow users to compute and analyze their metabolomics data easily and with very little sophisticated computer knowledge. The Metabolomics Workbench has 4 main goals: *1*) to act as a metabolomics data repository that helps to develop tools and interfaces for its users; *2*) to create a cloud computing infrastructure for metabolomics, thereby making the data transportable and easy to

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access; *3*) to coordinate with other metabolomics initiatives such as Regional Comprehensive Metabolomics Resource Cores, making the data comprehensive and centralized; and *4*) to provide continual resources that will help advance metabolomics research and education, which will in turn enable the development of new biomedicine.

Dr. Subramanian also provided several examples of applications of metabolomics data in systems biology and medicine. This includes work performed with the macrophage lipodome, through the Lipids Maps Consortium (2) where his group reconstructed the first lipidomic map of a mammalian cell (RAW 264.7 cells) under toll-like receptor 4 activation with LPS. This activation leads to changes in FA metabolism as well as delays in sphingolipid and sterol biosynthesis, which were detected by using quantitative MS. This line of research not only provided a systems-level view of lipid metabolism and remodeling but also showed connections between the lipids and different pathways that contribute to innate immune responses and to pharmacologic changes.

Dr. Fariba Assadi-Porter Discussed Metabolomics Applications in Polycystic Ovary Syndrome

Dr. Fariba Assadi-Porter is a Research Associate Professor in Nutritional Sciences at Texas Tech University.

Dr. Assadi-Porter gave an overview about the metabolomic dynamic platform (MDP)[3] that was developed in her laboratory. MDP couples several complementary technologies such as NMR, MS, cavity ringdown spectroscopy, and imaging, with broad applications to drug discovery, toxicology, nutritional research, and diagnostics. Her laboratory also uses stable isotopic labeling methodology to test and validate metabolic pathways that are predicted by the untargeted metabolomics. Dr. Assadi-Porter primarily focused on the application of metabolomics in women with polycystic ovary syndrome (PCOS) and its treatment by a natural hormone, 3-iodothyronamine.

Because data analysis is critical in metabolomics studies, Dr. Assadi-Porter's group has developed methods to semiautomate the data analysis. Through collaborations with biostatisticians and computational mathematicians, different statistical and algorithm methods were implemented to translate the metabolomics data into metabolic pathway interpretation. Automated methods were developed and implemented for NMR data collection from polysequencing and sample collection. Furthermore, MDP technology was applied to studying PCOS, a complex disease that is associated with endocrine disorders in 10–20% of women of reproductive age. There is no diagnosis for PCOS except by exclusion and only after puberty. Dr. Assadi-Porter hypothesized that higher androgen concentrations, inflammation, and insulin resistance (IR) all could contribute to PCOS, which results in this complex phenotype, and used untargeted metabolomics to test this hypothesis. In her recently published work, she used lean and obese non-PCOS subjects and lean and obese PCOS subjects (3). The NMR analyses of fasting serum data showed that the metabolite profiles were different between the 4 groups. Furthermore, glucose-tolerance tests demonstrated an inverse effect of glucose on some metabolites in women with PCOS than healthy women, independent of diabetes. These findings were further tested in a mouse PCOS metabolic model and validated with ^{13}C stable isotope labeling in order to trace and measure changes in their fluxes in both pentose phosphate and the Embden-Meyerhof pathway. This model showed that both pentose phosphate and glycolytic pathways contribute to the high rate of FA conversion, which contributes to metabolic dysfunction at the mitochondrial level.

Dr. James Ntambi Used Metabolomics to Complement Other Genetic and Biochemical Studies to Understand the Function of the Stearoyl CoA Desaturase

Dr. James Ntambi is a Katherine Berns Von Donk Steenbock Professor and Nutritional Sciences Department Chair at the University of Wisconsin–Madison.

Dr. Ntambi's presentation focused on the stearoyl-CoA desaturase (SCD) 1 (4), an endoplasmic reticulum enzyme that catalyzes the desaturation of SFAs, both palmitate (16:0) and stearate (18:0), at positions 9 and 10 to form palmitoleic (16:1n–7) and oleic (18:1n–9) acids, respectively. Both of them are major MUFAs that are found in TGs, cholesterol esters, and membrane phospholipids. The mouse *SCD* genome has 4 isoforms (*SCD1*, *SCD2*, *SCD3*, and *SCD4*), whereas the human genome has 2 isoforms (*SCD1* and *SCD5*). To understand the physiologic role of *SCD*, Dr. Ntambi's team generated global and tissue-specific knockout mouse models of *SCD1*.

The global knockout model was resistant to obesity with both diets despite hyperphagia. The liver *SCD1* knockout mice were not protected against high-fat diet–induced weight gain, but they were protected against high-carbohydrate diet–induced obesity and hepatic steatosis, due to the decreased de novo lipogenesis. Surprisingly, skin- but not adipose tissue– or liver-specific knockout mice were protected against high-fat diet–induced obesity. Because de novo lipogenesis is regulated by the transcription factor sterol regulatory element binding protein 1 (*SREBP-1*), *SCD1* gene inactivation dramatically reduced *SREBP-1* and lipogenic genes. To determine differential effects of the major *SCD1* products in hepatic lipogenesis and obesity, 2 transgenic mouse models were generated: the human *SCD5* (18:1n–9 product) and *SCD3* (16:1n–7) mouse models. The 2 models were crossed into the global knockout mice to specifically express the transgenes in the liver. When these mice were fed a high-carbohydrate diet or a low-fat diet, both 16:8n–9 and 16:1n–7 FAs were synthesized and incorporated into the liver specifically. Hepatic de novo lipogenesis in these transgenic mice (*SCD5* and *SCD3*) remained

[3] Abbreviations used: IR, insulin resistance; MDP, metabolomic dynamic platform; PCOS, polycystic ovary syndrome; SCD, stearoyl-CoA desaturase; SREBP-1, sterol regulatory element binding protein 1; T2D, type 2 diabetes.

low. In collaboration with Dr. Assadi-Porter, NMR-based metabolomics indicated a distinct metabolic profile in the *SCD1* global knockout mice than the wild-type or the transgenic mice that included changes in the metabolism of BCAAs, which are being further explored.

Dr. Sean Adams Presented on Metabolic Health, Host-Derived, and Xeno-Metabolite Profiles

Dr. Sean Adams is a Research Physiologist at the USDA Agricultural Research Service Western Human Nutrition Research Center and an Associate Adjunct Professor in the Department of Nutrition at the University of California, Davis.

Dr. Adams and collaborators use metabolomics as a tool to investigate the etiology of metabolic disease such as type 2 diabetes (T2D) and obesity with the goal of determining biomarkers that can be used as indicators for metabolic health in humans and in animal models. Major approaches used include metabolic profiling of muscle in T2D-relevant systems by using untargeted metabolomics technologies across several different platforms that are then used to generate working models and hypotheses. These models are then tested and validated at the cell and molecular level through an array of different techniques, including explorations in organelles, cells, and whole organisms.

Dr. Adams' group compared weight-matched obese subjects with or without T2D, which revealed that systemic BCAA concentrations correlated with glycated hemoglobin and elevated biomarkers of incomplete mitochondrial long-chain FA β-oxidation (5). From those metabolomics results, it was hypothesized that mitochondrial catabolism of both FAs and BCAAs, at least in some tissues, is dysfunctional under conditions of perturbed insulin action, and that this may be associated with "anaplerotic stress." After this, his group showed that branched-chain α-ketoacid dehydrogenase, the mitochondrial BCAA oxidation checkpoint, was decreased in obese white adipose tissue, which gave support to the hypothesis that in IR and T2D, BCAA utilization in white adipose tissue is also disrupted (6).

Dr. Adams also discussed metabolites that are "non-self" and come from dietary or gut microbial origin (xeno-metabolities) and how they are altered under different conditions such as T2D, IR, and improved metabolic fitness. Specifically, in studies in which diet was carefully controlled, they observed higher propane-1,2,3-tricarboxylate (tricarboxylic acid) after an oral-glucose-tolerance test and lower γ-tocopherol in an overnight-fasted state (7). This research provides examples of how peripheral blood or urinary signatures of metabolic health can be influenced by gut-derived metabolites. This demonstrates that host-gut microbiome communication is a 2-way street, in that gut-derived signals can alter host physiology but also that host metabolic health status seems to impact gut microbe biology and metabolism independent of diet.

In summary, this symposium provided a comprehensive overview of metabolomics tools and applications to identify metabolic signatures specific to metabolic diseases or nutritional conditions. This technology holds promise in expediting our ability to develop targeted preventive and treatment strategies (both nutritional, pharmacologic, and genetic) for chronic diseases.

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