Metabolic Profiling in Nutrition and Metabolic Disorders1,2

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ABSTRACT

Nutrients exert potent effects on metabolism through a variety of regulatory mechanisms, resulting in local and systemic changes in metabolite levels. Numerous studies have focused on mechanisms by which nutrients and disease states regulate metabolism at the gene or protein levels using genomic and proteomic approaches, respectively. However, few studies have investigated nutritional regulation of the whole metabolome. Thus, metabolomic approaches have recently emerged to complement the genomics and proteomics research and to help identify biologically meaningful metabolites and metabolic networks that control cellular responses to genetic and environmental factors, including diet, and to identify metabolic diseases that are influenced by genetic and dietary factors. These large-scale studies expedite our ability to develop targeted treatments. The goal of this symposium was to provide a forum to introduce the metabolomics field to nutrition researchers. An overview of the state-of-the-art metabolomic technologies used was provided. The impact of some specific nutrients, disease states, or genetic variations and their interaction with the metabolome was discussed by the speakers. Our objectives were as follows: 1) to educate the audience about the use of metabolomics as an innovative tool for linking changes in cell metabolites and genetic variations to nutrient metabolism, energy balance, and the overlying effects on health and disease; 2) to understand the concept of metabolomics and describe the analytical tools and resources available in this area; 3) to introduce the potential application of metabolomics in the field of nutrition research; and 4) to provide specific nutrition-relevant metabolomics study examples in investigating regulation of the metabolic network or metabolic changes resulting from disease states by dietary factors. Adv. Nutr. 4: 548–550, 2013.

Introduction

Health and disease states are tightly controlled by genetic and environmental conditions. Nutrients exert potent effects on metabolism through regulatory mechanisms at the transcriptional, posttranscriptional, translational, or posttranslational levels, resulting in changes at the tissue and/or systemic metabolite levels. Numerous studies have addressed regulation of protein and gene expression using a variety of “omics” tools. However, few studies have investigated the nutritional regulation of the whole metabolome.

Only recently has the field of metabolomics emerged to complement the genomics and proteomics fields. Metabolomics studies offer the advantage of linking nutrient-gene interactions to specific metabolites and small molecules. Such studies help identify biologically meaningful metabolic networks that control cellular responses to environmental factors, including diet, and identify metabolic diseases that are influenced by nutrients.

Metabolomic approaches allow a comprehensive profiling of the cell metabolome or “library of metabolites” that provides chemical signatures of cell dynamics and metabolic activity. Such comprehensive studies of metabolic alterations in disease conditions enhance and expedite our ability to develop targeted treatments.

Various analytical approaches are used to identify metabolites. The major approaches in metabolomics studies include MS- and NMR-based techniques. Before detection of metabolites using MS-based techniques, samples are separated by using many methods including GC, high performance liquid chromatography (HPLC), and capillary electrophoresis (CE)3.

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3 Abbreviations used: CE, capillary electrophoresis; CHDH, choline dehydrogenase; DI, direct infusion; LC, liquid chromatography; MALDI, matrix-assisted laser desorption/ionization; MCK, muscle creatine kinase; MTHFD1, methylenetetrahydrofolate dehydrogenase 1; PGC1α, phosphatidylethanolamine N-methyltransferase; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1α; PPAR, peroxisome proliferator-activated receptor; SAM, S-adenosylmethionine; SFC, supercritical fluid chromatography; SNP, single nucleotide polymorphism; TCA, tricarboxylic acid.
GC is used to separate volatile metabolites, such as organic acids and fatty acids, and HPLC is best suited for soluble and lipophilic metabolites. MS can detect metabolites quantitatively and qualitatively. After extracting from biological samples, the metabolites are split equally into 2 parts, which are used for analysis on the GC/MS and liquid chromatography (LC)/MS platforms, respectively. After separation, metabolites are ionized and then sorted on the basis of the mass-to-charge ratios by a mass analyzer. Finally, an MS detector identifies and quantifies the metabolites. MS can also identify unknown compounds and determine their chemical structure. The advantages of MS-based techniques are high sensitivity and specificity. NMR, on the other hand, does not require metabolite separation, and it can measure all metabolites simultaneously. The advantages of NMR are simple sample preparation and high analytical reproducibility, but it has lower sensitivity than MS-based techniques. Even though NMR, LC/MS, and GC/MS are commonly used techniques in metabolomics, other methods that have been used are CE/MS, supercritical fluid chromatography (SFC)/MS, matrix-assisted laser desorption/ionization (MALDI)/MS, and direct infusion (DI)/MS.

### Summary of the Symposium

The goal of this symposium was to provide a forum to introduce nutrition researchers to the metabolomics field. This was accomplished by providing attendees an overview of the state-of-the-art metabolomic technologies used. Specific applications to study how nutrients, disease states, or genetic variations interact to alter the metabolome were discussed by the speakers. Specifically, the objectives of this symposium were as follows:

1. Educate the audience about the use of metabolomics as an innovative tool for linking changes in cell metabolites and genetic variation to nutrient metabolism, energy balance, and the overlying effects on health and disease;
2. Understand the concept of metabolomics and describe the analytical tools and resources available in this area;
3. Understand how metabolomics approaches could be implemented in the field of nutrition;
4. Provide specific nutrition-relevant examples of how metabolomics are used to study nutrient regulation of the metabolic network or metabolic changes resulting from disease states.

The presentations by each speaker are summarized below.

**Steven H. Zeisel, MD, PhD, Kenan Professor of Nutrition and Pediatrics and Director of the Nutrition Research Institute and the Clinical Nutrition Research Center at The University of North Carolina, Chapel Hill.** Dr. Zeisel’s presentation was entitled “Metabolomic Profiling in People with Nutritionally Relevant SNPs.” He used untargeted metabolomic profiling to determine dietary choline requirements for humans. This was carried out by the company Metabolon, Inc., which uses a combination of GC/MS and LC/MS/MS (as described above) for metabolomic profiling.

Dr. Zeisel presented results from a clinical study in which participants received a controlled diet that contained choline for 10 d. Choline was then reduced, resulting in most of the participants developing fatty liver. This, along with other symptoms, was reversed when they were fed a diet containing choline. By the end of the study, most of the male and postmenopausal female participants developed either liver or muscle damage due to choline deficiency.

However, only 44% of the premenopausal females developed these phenotypes. This was likely due to the estrogen-induced activation of the phosphatidylethanolamine N-methyltransferase (PEMT) gene, responsible for the synthesis of choline in the liver, which therefore protected these participants from damage. To understand why 44% of premenopausal women were sensitive to choline deficiency, Dr. Zeisel identified several single nucleotide polymorphisms (SNPs) that are common in choline metabolism genes. Of importance were SNPs identified in 3 genes: PEMT, choline dehydrogenase (CHDH), and methylenetetrahydrofolate dehydrogenase 1 (MTHFD1). The SNP in PEMT prevented estrogen from activating the PEMT gene, thereby inhibiting choline production, and resulted in a 25-fold increased risk of becoming choline deficient. CHDH is involved in the conversion of choline into betaine, leading to women with an SNP in this gene to have a 20-fold increased risk of choline deficiency. Last, the SNP in the MTHFD1 gene was shown to restrict methyl group availability, and thereby S-adenosylmethionine (SAM) availability, which are needed for PEMT formation of choline. In general, this SNP was shown to result in an 85-fold increased risk in both in men and women when choline becomes low in the diet. In addition to identifying SNPs, metabolomic profiling was also used to investigate plasma markers after consumption of a normal diet, a depletion diet, and a choline-repleted diet, as well as to identify specific signatures for these diets. These studies indicated that several metabolites and metabolic processes were regulated by choline, including carnitine metabolism and mitochondrial function and amino acid metabolism.

**Robert E. Gerszten, PhD, Center for Immunology and Inflammatory Diseases, Massachusetts General Hospital (MGH); Director of the Clinical and Translational Research Program, MGH Heart Center; Associate Professor of Medicine, Harvard Medical School and Senior Associate, Broad Institute of Harvard and Massachusetts Institute of Technology.** Dr. Gerszten’s presentation was entitled “Metabolomics and Cardiovascular Biomarker Discovery.” He used targeted metabolomics approaches to study the molecular basis of injury responses in cardiovascular disease, mainly through the use of a triple quadrupole LC/MS. Initially, this technique was used to identify early markers of myocardial injury that occur within the first few minutes postinjury. This was important because most standard biochemical markers take hours before they can be used to indicate a myocardial injury. Although Dr. Gerszten was able to detect numerous changes...
in metabolites just 10 min postinjury, these results were in patients who had undergone a planned myocardial injury experiment.

Therefore, to obtain a more “natural” validation of their technique, Dr. Gerzsten and his group decided to examine samples from the Framingham Heart Study that were collected 12 y ago to see if they could detect metabolites that correlate with cardiovascular disease before its onset. During this analysis, they identified several branched amino acids that were increased and correlated positively with the Framingham Heart Study cardiovascular disease phenotypes.

Branched amino acids have also been connected with diabetes and in metabolism signatures that differentiate obese and lean populations, making them of particular interest for biomarkers in cardiovascular disease.

Dr. Moustaid-Moussa, PhD, Professor of Nutritional Sciences and Leader of The Obesity Research Cluster at Texas Tech University. Dr. Moustaid-Moussa’s presentation was entitled “Metabolic Profiling of Omega-3 Fatty Acid Effects in High Fat Fed Mice.” She discussed recent work from her laboratory where they investigated whether EPA can prevent and reverse diet-induced obesity, inflammation, and insulin resistance. In the study, they used a combination of genomic and metabolomic approaches to further investigate the molecular basis for these effects.

Physiologic and molecular studies showed that feeding mice high-fat diets (45% of energy) induced obesity, glucose intolerance, hepatic steatosis, and insulin resistance, all of which were prevented when the high-fat diet was enriched with omega-3 (n-3) fatty acids. These changes were at least in part related to improvements in systemic and adipose inflammation.

Remodeling of adipose tissue was associated with decreased fat cell size in the EPA-fed mice, consistent with decreased adipogenic markers and lipid accumulation. Metabolomic analyses, performed by Metabolon, Inc., confirmed an enrichment of n-3 PUFAs and a decrease in n-6 PUFAs in adipose tissue. However, changes in the various metabolites related to glucose, amino acid, and lipid metabolism were tissue specific.

For example, polyamine metabolites such as spermidine were increased in adipose tissue and were accompanied with decreased adipocyte differentiation. Conversely, branched-chain amino acids were increased in the muscle, whereas biomarkers of lipid metabolism and oxidative stress were mainly altered in the liver. These metabolic changes were in line with other genomic, metabolic, and physiologic outcomes, including decreased adiposity and fat cell size and increased expression and metabolite amounts in lipid oxidation pathways and decreased expression of genes and proteins in fatty acid and TG synthesis pathways. Further directions include exploring additional mechanisms mediating EPA effects in target tissues (adipose, muscle, liver, and pancreas) and testing whether n-3 fatty acids can prevent the development of diabetes and insulin resistance in humans with prediabetes or early type 2 diabetes.

Deborah M. Muoio, PhD, Associate Professor in the Sarah W. Stedman Nutrition and Metabolism Center, Departments of Medicine and Pharmacology and Cancer Biology, Duke University. The title of Dr. Muoio’s talk was “Metabolomics and Muscle Metabolism in Metabolic Syndrome.” Integrating genomics, proteomics, and targeted metabolomics approaches together, she focused on mitochondrial health in obesity and aging and the effects of exercise on both. During her presentation, Dr. Muoio discussed a collaboration between her group and the STRRIDE (Studies of a Targeted Risk Reduction Intervention through Defined Exercise) study, which evaluates the effectiveness of exercise alone vs. exercise plus diet intervention in sedentary, overweight adults with prediabetes. Through this collaboration, Dr. Muoio showed that exercise training increased muscle amounts of fatty acid–derived acylcarnitines metabolites and tricarboxylic acid (TCA) cycle intermediates and therefore increased β-oxidation. In turn, these fatty acid–derived acylcarnitines, but not other metabolites, correlated with expression of lipid-related genes. However, these metabolite signatures were quite variable, with exercise-induced improvements in insulin sensitivity varying between and within groups.

Dr. Muoio also discussed some of the animal work conducted in her laboratory, which targets mitochondrial manipulations in order to understand the relationship between pathways such as fatty acid oxidation or acylcarnitine metabolism and insulin action. Of particular interest is their work with transgenic mice that overexpress peroxisome proliferator-activated receptor (PPAR)-γ coactivator 1α (PGC1α) in skeletal muscle, driven by the muscle creatine kinase (MCK) promoter (MCK-PGC1α mice). These mice are known to have increased oxidative metabolism in muscle while still displaying increased susceptibility to diet-induced insulin resistance. A look at the metabolic signature of these transgenic mice revealed that it resembled those of exercise-trained humans. However, a mere 2 wk of high-fat feeding was able to disrupt the acylcarnitine/acyl-CoA balance in these animals. Dr. Muoio hypothesized that this disruption might contribute to the observed insulin resistance, and it is a focus of possible future studies.

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