Metabolomics and Exposome Laboratory Service Core (MEL)
University of North Carolina at Chapel Hill
Director: Susan Sumner, PhD
Assistant Director, Blake Rushing, PhD

Overview: The Metabolomics and Exposome Laboratory (**MEL**) is located at the UNC Nutrition Research Institute and is directed by Dr. Susan Sumner, who is a professor of nutrition and pharmacology at UNC Chapel Hill. The methods employed by the MEL were developed through grants from the NIH Common Fund Phase 1 (U24DK097193) and Phase 2 (U01CA235507) Metabolomics Program, the NIEHS Children's and Human Health Exposure Analysis Resource (CHEAR, HHEAR) programs and the Environmental influences on Childhood Outcomes (ECHO) program (U2CES026544, U2CES030857), and are being expanded through funding as the Metabolomics and Clinical Assay Center (MCAC) in the NIH Common Fund Nutrition for Precision Health (NPH) study (5U24CA268153).

The MEL offers a wide range of services to support basic, clinical, and translational research in areas of precision medicine, nutrition, and environmental health. Using state-of-the-art metabolomics and exposome technologies, our team determines how molecules that are present in our tissues and biological fluids are associated with states of health and wellness, and response to treatment and intervention. Through this approach, biomarkers are discovered that can lead to new diagnostics for the early detection and diagnosis of disease, to monitor treatment and intervention, and to inform the development of new intervention strategies. Investigations are also conducted using *in vitro* and *in vivo* model systems to reveal the mechanistic underpinnings of disease or response to treatment, and to provide translational approaches.

Biospecimen Types: We have experience with a range of biospecimens (e.g., urine, plasma, serum, stool, cecal, breast milk, organ tissues and cell extracts) in studies of osteoarthritis, cancer, obesity, liver disease, kidney disease, eye disease, infection, pregnancy complications, nutrient and dietary intake, and addiction.

MEL Services:

- **Service 1: Consultation**
    - MEL experts are available to advise on study design, sample selection, biospecimen collection and storage, data analysis approaches, and proposal or manuscript development.
- **Service 2: Broad Spectrum Quantitative Targeted Analysis for up to 188 Analytes of Host and Microbial Metabolism**.
    - This analysis uses the standardized AbsoluteIDQ® p180 kit from Biocrates Life Sciences. Data is acquired using a state-of-the-art Waters TQ-XS triple quadrupole mass spectrometry platform, which is in the class of the most sensitive and selective quantitative MS instruments on the market today.
    - The analysis provides up to 188 metabolites from 5 compound classes including amino acids (21), biogenic amines (21), acylcarnitines (40), glycerophospholipids (90), sphingolipids (15) and hexoses (1). For a list of analytes, click here biocrates-p180-list-of-metabolites-v2-2021.pdf
- **Service 3: Broad Spectrum Quantitative Targeted Analysis for up to 630 Analytes of Host and Microbial Metabolism**.
    - This analysis uses the standardized MxP® Quant 500 kit from Biocrates Life Sciences. Data is acquired using a state-of-the-art Waters TQ-XS triple quadrupole mass spectrometry platform, which is in the class of the most sensitive and selective quantitative MS instruments on the market today.
    - Broad metabolite coverage of up to 630 metabolites from 26 biochemical classes including alkaloids (1), amine oxides (1), amino acids (20), amino acid related (30), bile acids (14), biogenic amines (9), carboxylic acids (7), cresols (1), fatty acids – free/non-covalently bound (12), hormones and related (4), indoles and derivatives (4), nucleobases and related (2), vitamins and cofactors (1), carbohydrates and related (1), acylcarnitines (40), lysophosphatidylcholines (14), phosphatidylcholines (76), sphingomyelins (15), ceramides (28), dihydroceramides (8), hexosylceramides (19), dihexosylceramides (9), trihexosylceramides (6), cholesteryl esters (22), diglycerides (44) and triglycerides (242). For a list of analytes, click here biocrates-Quant500-list-of-metabolites-v6-2022.pdf.

- **Service 4: Quantitative Targeted Analysis of One Carbon Metabolism**
    - Quantitative targeted analysis of choline and choline-related metabolites are conducted using a Xevo TQD and TQSμ Triple Quadrupole Mass Spectrometers (Waters, Wilmslow, Manchester, UK).
    - This includes choline, betaine, phosphatidylcholine, phosphocholine, glycerophosphocholine, sphingomyelin, TMA, TMAO, and creatinine.
- **Service 5: Untargeted Analysis of Host, Microbial, and Exposures via High Resolution Mass Spectrometry**
    - Untargeted analysis is conducted using ultra-high performance liquid chromatography (UHPLC) coupled with a Thermo Fisher Q-Exactive HF-X or IQ-X™ Mass Spectrometer.
    - Our method enables the detection of 10s of 1000s of signals in biospecimens.
    - We have developed an in-house Retention Time (RT), Exact Mass, and MS/MS fragmentation library by acquiring data for over 2,500 compounds on the untargeted platform. The range of analytes that we know can be detected on the UHPLC-HR-MS system and that have been detected in human biospecimens include the following. *Host Metabolism*: We have acquired data for over 1,200 standards that represent the host metabolism: including amino acids, carboxylic acids, biogenic amines, polyamines, bases, nucleosides and nucleotides, carnitines, sugars, mono- and disaccharides, fatty acids, lipids, steroids, bile acids, neurotransmitters, and hormones. In addition, we acquired data for standards associated with one carbon metabolism, folate metabolism, and vitamins and vitamin-like compounds due to their importance as serving as the co-factors for biochemical processes. *Environmentally Relevant Analytes*: Classes of environmentally relevant analytes are simultaneously detected with the host and microbial metabolites on our untargeted platform. We have acquired data for over 600 metabolites of alkyl phosphate pesticides, phthalates, polycyclic aromatic hydrocarbons, volatile organic compounds (VOCs), perfluoro compounds, metabolites of environmental phenols, and parabens. Some of the related parent compounds of these metabolites may be ingested with foods (e.g. parabens) or beverages (e.g., phthalates), as well as come from other sources of environmental contamination. Others are derived from tobacco products (e.g., cotinine), medications (e.g., acetaminophen, metformin), and illicit drugs (e.g., heroin, morphine, opioid metabolites). Metabolites derived from medications may find relevance not only from prescription or over the counter use, but also because of the introduction of antibiotics in the food chain through for example the poultry and livestock industry (e.g., sulphaguanidine), or the use of medications which can inhibit metabolic processes of host metabolism. Additionally, some of the VOCs that are detected are known metabolites to be derived from the combustion of plant matter, such as tobacco smoke, foods heated to high temperature, or wood smoke. *Microbial Co-Metabolites:* The current library has about 200 microbial co-metabolites. The gut microbiome and the potential role of microbial metabolites in human health and disease have garnered significant attention in recent years, and hold promise in development of biomarkers. *Metabolites from Ingested Foods*: We have already acquired data for over 500 standards, from 50 subclasses, that were shown to increase in biospecimens obtained from 10 different human feeding intervention studies. The initial analysis was conducted for those 10 human feeding intervention trials using UPLC-MS(n) quantitative methods (over 5,000 biospecimens) and has resulted in the identification of these compounds. The library continuously expands.
    - We use algorithms to match signals to the in-house physical standards library, and to 1.7M public database spectra.

- **Service 6: NMR Metabolomics:**
    - We acquire data using a Bruker Avance III 700 MHz NMR spectrometer.
    - Metabolomics data can be provided via spectral binning, or by concentration fitting signals to a pH sensitive library of over 350 compounds.

- **Service 7: Structure elucidation, and quantitation**

- NMR spectroscopy is a powerful technique for structure elucidation of both known or unknown molecules using 1D and 2D/multidimensional techniques such as COSY, HSQC, HMBC, TOCSY, and HSQC-TOCSY. We have experience in the elucidation of metabolite structures and the development of quantitative methods using authentic standards or material with similar structure features for quantitative estimates.

- **Service 8: Isotopic Tracing:**
  - We have experience in tracing the fate of ^{13}C labeled compounds following the treatment of cells or administration of labeled material to rodents or humans.

- **Service 9: Human and Rodent Cytokine Analysis**
  - High-throughput, relative quantitation analysis using the RayBiotech arrays to acquire data for 42 or 80 cytokines in human samples, 62 cytokines in mouse samples, and 34 cytokines in rat samples.

- **Service 10. Statistics, Multivariate Analysis, and Modelling**.
  - Descriptive statistics and hypothesis testing are conducted using R or SAS 9.4 (SAS Institute Inc., Cary, NC). Unsupervised (e.g., principal component analysis) and supervised (e.g., orthogonal partial least squares discriminate analysis, OPLS-DA) multivariate analysis of data are conducted using SIMCA 17.0 (Sartorius Stedim Data Analytics, Umeå, Sweden). Bins with VIP ≥ 1 with low variance, or p-value and fold change of significance are considered important for differentiating study phenotypes. The Variable Importance to Projection (VIP) statistic from a PLSDA or OPLS-DA analysis is provided as the importance of the metabolites in differentiating the phenotypic groups. All models use a 7-fold cross-validation to assess the predictive variation of the model (Q2). Regression modelling can be performed using subject characteristic data, metabolite or bin data, and study outcome.
  - Faculty, staff, and students can receive training in data analysis.

- **Service 11. Pathway and Multi-omics Analysis:**
  - MetaCore software (Clarivate Analytics, PA) or Metaboanalyst are used for pathway enrichment analysis, and pathways are ranked by *p*-value based on the hypergeometric test, which represents the enrichment of certain metabolites.
  - Multi-omics data analysis is conducted by combining metabolomics with other omics (e.g., proteomics) using Metacore or Metaboanalyst.
  - Faculty, staff, and students can receive training in data analysis.

- **Service 12: Special Methods**
  - The MEL can develop new methods under a time and material agreement.

Location and Biospecimen Delivery

The Metabolomics and Exposome Laboratory is located at the UNC Chapel Hill Nutrition Research Institute on the North Carolina Research Campus in Kannapolis, North Carolina.

Samples can be shipped to the MEL via Fed Ex or another certified courier service.

Contact Blake Rushing (blake_rushing@unc.edu) and Sabrina Molina (sabrina_molina@unc.edu) for shipment instructions.

MEL Faculty

Susan Sumner, PhD: https://uncnri.org/faculty-susan-j-sumner-phd/

Blake Rushing, PhD: https://uncnri.org/staff-members/blake-rushing-phd/

Wimal Pathmasiri, PhD: https://uncnri.org/staff-members/wimal-pathmasiri-phd/

Contact the MEL Faculty and Staff

Service	Role	Name	Email
Service 1 Consultation	Director, MEL Program Manager	Susan Sumner, PhD Susan McRitchie, PhD	Susan_sumner@unc.edu Susan_mcritchie@unc.edu
Services 2 &3 Biocrates Analysis	Quantitative Targeted Analysis of Host and Microbial Metabolism	Rachel Coble, BS Susan Sumner, PhD	rachel_coble@unc.edu susan_sumner@unc.edu
Service 4 Choline Metabolism	Quantitative Targeted Analysis of One Carbon Metabolism	Rachel Coble, BS Susan Sumner, PhD	rachel_coble@unc.edu susan_sumner@unc.edu
Service 5 Untargeted Metabolomics via High Resolution Mass Spectrometry	Untargeted Analysis of Host/Microbial metabolism, and Exposures via High Resolution Mass Spectrometry	Blake Rushing, PhD Sabrina Molina, BS	Blake_rushing@unc.edu sabrina_molina@unc.edu
Service 6 NMR Metabolomics	NMR Metabolomics via binning or concentration analysis	Wimal Pathmasiri, PhD Blake Rushing, PhD	Wimal_pathmasiri@unc.edu Blake_rushing@unc.edu
Service 7 Structure elucidation, and quantitation	Metabolite identification and quantitation	Wimal Pathmasiri, PhD Blake Rushing, PhD	Wimal_pathmasiri@unc.edu Blake_rushing@unc.edu
Service 8 Isotopic Tracing	Tracing 13C isotopic labels	Wimal Pathmasiri, PhD Blake Rushing, PhD	Wimal_pathmasiri@unc.edu Blake_rushing@unc.edu
Service 9 Cytokine Analysis	Human and Rodent Cytokine Analysis	Susan Sumner, PhD Blake Rushing, PhD	Susan_sumner@unc.edu Blake_rushing@unc.edu
Service 10 Data Analysis	Statistics, Multivariate Analysis, and Modelling	Susan McRitchie, MS Susan Sumner, PhD	Susan_mcritchie@unc.edu Susan_sumner@unc.edu
Service 11 Pathway Enrichment Analysis	Biochemical Interpretation	Wimal Pathmasiri, PhD Susan Sumner, PhD	Wimal_pathmasiri@unc.edu Susan_sumner@unc.edu
Service 12 Special Methods	Targeted analysis of selected analytes or pathways	Susan Sumner, PhD Blake Rushing, PhD	Susan_sumner@unc.edu Blake_rushing@unc.edu