Metabolic Profiling of Blood and Urine for Exploring the Functional Role of the Microbiota in Human Health

Biological Research for Nursing
2020, Vol. 22(4) 449-457
© The Author(s) 2020
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1099800420941080
journals.sagepub.com/home/brn

Ana F. Diallo, PhD, MPH, RN¹ , Mark B. Lockwood, PhD, RN²,
Katherine A. Maki, PhD, MS, AGNP-BC² , Alexis T. Franks, BS³ ,
Abhrrarup Roy, BS³, Rosario Jaime-Lara, PhD, MS, FNP-BC³ ,
Paule V. Joseph, PhD, MS, FNP-BC, CTN-B³ ,
Wendy A. Henderson, PhD, MSN, CRNP, FAAN⁴ ,
Seon Yoon Chung, PhD, RN⁵,
Jacqueline McGrath, PhD, RN, FNAP, FAAN⁶,
Stefan J. Green, PhD⁷ , and Anne M. Fink, PhD, RN, FAHA²

Abstract

The quantification of metabolites in blood and urine allows nurses to explore new hypotheses about the microbiome. This review summarizes findings from recent studies with a focus on how the state of the science can influence future nursing research initiatives. Metabolomics can advance nursing research by identifying physiologic/pathophysiologic processes underlying patients' symptoms and can be useful for testing the effects of nursing interventions. To date, metabolomics has been used to study cardiovascular, respiratory, endocrine, autoimmune, and infectious conditions, with research focused on understanding the microbial metabolism of substrates resulting in circulating/excreted biomarkers such as trimethylamine N-oxide. This review provides specific recommendations for the collection of specimens and goals for future studies.

Keywords

blood, cardiovascular, diabetes, trimethylamine n-oxide, metabolomics, microbiome, nursing, urine

The microbiome, the collection of microorganisms living in the body along with their genetic material, affects health and disease. Most research on this topic has focused on defining the microbial species residing in the gastrointestinal tract and determining the influence of these microbes on host metabolic functions (Huttenhower et al., 2012). An impaired microbiome (dysbiosis), which may be the result of reduced microbial diversity, can adversely affect metabolic processes in the host (Daliri et al., 2017). When microbes metabolize food or medications in the colon, the resulting metabolites enter the circulation and can be quantified in the blood and urine. By analyzing the *metabolome*, the complete set of metabolites in the circulation, researchers gain valuable insights into cellular processes that cannot be determined by studying the microbiome alone.

Bacteria in the colon ferment carbohydrates to produce succinate and short-chain fatty acids (e.g., butyrate, acetate, propionate; Bugaut, 1987). Succinate, for example, activates immune cells in the mucosal layer of the intestine and enhances inflammation (Connors et al., 2018). Butyrate, however, plays an important protective role in maintaining the epithelial barrier (Baxter et al., 2019). Protein catabolism is also regulated by

¹ Institute of Inclusion, Inquiry & Innovation (iCubed), Family and Community Health Nursing, School of Nursing, Virginia Commonwealth University, Richmond, VA, USA

² Department of Biobehavioral Health Science, College of Nursing, University of Illinois at Chicago, Chicago, IL, USA

³ Sensory Science & Metabolism Unit, Biobehavioral Branch, Division of Intramural Research, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA

⁴ Digestive Disorders Unit, Biobehavioral Branch, Division of Intramural Research, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA

⁵ School of Nursing, University of Maryland, Baltimore, MD, USA

⁶ School of Nursing, University of Texas Health Science Center, San Antonio, TX, USA

⁷ Sequencing Core, Research Resources Center, University of Illinois at Chicago, Chicago, IL, USA

Corresponding Author:

Ana F. Diallo, PhD, MPH, RN, Institute of Inclusion, Inquiry & Innovation (iCubed), Family and Community Health Nursing, School of Nursing, Virginia Commonwealth University, Richmond, VA, USA.

Email: dialloa@vcu.edu

gastrointestinal microbes, resulting in the biosynthesis of organic compounds such as trimethylamine N-oxide (TMAO) and indolepropionic acid (IPA). TMAO is derived from dietary precursors, including choline, carnitine, creatinine, betaine, and lecithin, and has a hypothesized role in dysbiosis (Chhibber-Goel et al., 2017; Nowinski & Ufnal, 2018; Velasquez et al., 2016). IPA, a microbial metabolite of tryptophan, exerts antioxidant and anti-inflammatory effects on the intestinal epithelium (Anesi et al., 2019; Pavlova et al., 2017; Tuomainen et al., 2018).

Researchers have examined the fecal microbial composition to classify the gut as healthy or unhealthy. When the integrity of the intestinal wall is compromised by inflammation, cells and bacterial fragments leak from the intestine into the circulation and activate systemic immune and inflammatory pathways. If researchers only examine characteristics of the gut microbiome, their studies lack information about the inflammatory and metabolic consequences occurring in the circulation. Considering this limitation, there is a need for methodological recommendations for assessing the metabolome. Recommendations specific to nursing have focused on fecal microbiome methods (Maki et al., 2018), but advances in the analysis of blood and urine must now be addressed. The collection of blood or urine for research is feasible, and metabolic profiling can be used to explore hypotheses about the relationships among gastrointestinal microbes, metabolic pathways, and immune/inflammatory mechanisms (Wolfe & Brubaker, 2019).

A variety of methods can be used to quantify metabolites, including mass spectroscopy, nuclear magnetic resonance (NMR), and high-performance liquid chromatography (HPLC); the specific analytic methods are beyond the scope of this review. Considering the important role of metabolomics in advancing nursing science, the purpose of this review is to synthesize the findings of recent studies about blood and urine metabolomics and to provide researchers with methodological recommendations specific to future nursing research initiatives.

Methods

We searched the NCBI/PubMed database for data-based publications in English using the search terms “*urine, plasma, OR serum*” and “*microbiome OR metabolite*” to identify relevant studies about human health or disease processes. Only studies focused on human health/disease that were published within the last 3 years were considered for review. Considering that metabolomics is a rapidly evolving field with significant advances in technologies and analytic methods reported over the past 3 years, we did not include articles published before 2017.

As illustrated in Figure 1, we retrieved 518 articles from the initial database search. We excluded 152 studies that did not involve human subjects and/or were not published within the last 3 years and then screened the remaining 366 articles by reviewing titles and abstracts. We excluded an additional 279 articles as duplicates, primers, or editorials. Finally, after full-text review, we removed an additional 66 articles because they

were methodological/review papers that did not report original research.

Results

We selected 21 data-based studies testing plasma, serum, or urine for review. The studies examined cardiovascular (Amin et al., 2019; Hayashi et al., 2018; Liu et al., 2018; Matsuzawa et al., 2019; Nie et al., 2018; Walejko et al., 2018; Zhai et al., 2019; Zhu et al., 2020), respiratory (Ottiger et al., 2018), and autoimmune (Bellocchi et al., 2018) disorders as well as diabetes (Li et al., 2018; Liu, Ling, et al., 2017; Tuomainen et al., 2018; Urpi-Sarda et al., 2019), obesity (Liu, Hong et al., 2017; Ottosson et al., 2018; Serena et al., 2018), and human immunodeficiency (HIV) infection (Missailidis et al., 2018; Moon et al., 2018; Shan et al., 2018; Wang et al., 2019).

Blood Testing

Reviewed studies ($N = 21$) measured plasma or serum metabolic markers, focusing on how various disease states may alter metabolic and inflammatory pathways (Table 1). Some ($n = 9$) also included fecal samples, which allowed researchers to explore correlations between metabolic profiles and gastrointestinal bacterial characteristics (Bellocchi et al., 2018; Hayashi et al., 2018; R. Liu et al., 2017; Missailidis et al., 2018; Moon et al., 2018; Ottosson et al., 2018; Serena et al., 2018; Walejko et al., 2018; Wang et al., 2019). Several ($n = 12$), however, only provided data about the metabolites detected in the blood without collecting data that would allow for the interpretation of the findings in the context of the fecal microbiome.

Several studies identified elevated circulating TMAO levels as a marker of worsening cardiovascular and endocrine disease processes. For example, plasma TMAO levels were associated with vulnerable plaque characteristics in the coronary arteries (Liu et al., 2018). After a nonST-segment myocardial infarction, chronically elevated plasma TMAO levels predicted future cardiovascular events (median follow-up of 5 years; Matsuzawa et al., 2019). Serum and plasma TMAO levels were associated with the risk for stroke in hypertensive patients (Nie et al., 2018) as well as adverse outcomes following stroke, such as cognitive impairments (Zhai et al., 2019; Zhu et al., 2020). Increased plasma TMAO levels predicted all-cause mortality in patients with chronic obstructive pulmonary disease, but the association was not significant after controlling for comorbidities and age (Ottiger et al., 2018). Patients with heart failure had significantly higher plasma TMAO levels than healthy subjects, and TMAO levels were associated with a higher fecal abundance of the *Escherichia/Shigella* genera in heart failure (Hayashi et al., 2018). Plasma TMAO levels were also used in clinical trials to assess the risk for gestational diabetes (Li et al., 2018). Overall, these data have led researchers to consider a potential atherosclerotic role for this molecule in the pathophysiology of cardiovascular and endocrine disorders.

Studies examining the metabolism of tryptophan suggest a protective role for this amino acid, unlike TMAO. Tuomainen

Figure 1. PRISMA (Preferred Reporting Items for Systemic Reviews and Meta-Analyses) Diagram of Study Selection Process.

et al. (2018) tested the hypothesis that serum levels of IPA, a metabolite of tryptophan, were associated with a reduced risk of developing Type 2 diabetes (T2D). IPA levels were positively correlated with insulin secretion and dietary fiber intake and inversely correlated with the inflammatory biomarker C-reactive protein. These observations led the authors to suggest that relationships among IPA, β -cell function, and inflammation may be influenced by dietary fiber intake (Tuomainen et al., 2018).

In addition to TMAO and IPA, researchers have explored other potential biomarkers to discover pathophysiologic mechanisms. Based on an exploratory NMR-based analysis of plasma metabolites, Amin et al. (2019) reported potential abnormalities in urea, ketone, and methylhistidine metabolism in patients with coronary artery disease. The study was not

designed, however, to link plasma markers with specific microbiome characteristics. In another study, investigators identified a unique metabolic profile in African American patients with high blood pressure (Walejko et al., 2018). The profile was characterized by elevated plasma levels of sulfacetaldehyde, quinolinic acid, 5-aminolevulinic acid, leucine, and phenylalanine in addition to lower levels of 4-oxoproline and L-anserine. The researchers suggest that alterations in the gastrointestinal microbiome could augment inflammation, oxidative stress, and insulin resistance and that different microbiome and metabolic profiles exist in hypertensive patients by race, a hypothesis that remains to be tested in future studies.

Other studies have examined obesity-related changes in microbial species and their metabolic consequences. In a metagenome-wide association study of lean and obese subjects,

Table 1. Characteristics of Selected and Reviewed Studies (N = 21).

Authors/Year	Disease/ Symptoms	Metabolite/ Microbial Samples	Analysis Method	Metabolite/Metabolic Pathways	Main Outcomes
Amin et al., 2019	CAD	Plasma/ urine	NMR	Significant disturbances in metabolic pathways in patients with CAD, including enrichment of the methylhistidine metabolism pathway and perturbations in galactose and histidine metabolism	Not investigated
Bellocchi et al., 2018	Systemic sclerosis	Plasma/fecal	16 S rRNA gene amplicon sequencing/ HPLC-MS	Higher levels of glycerophospholipid metabolites in patients with systemic sclerosis compared to healthy controls	Overrepresentation of <i>Parabacterioides</i> , <i>Firmicutes</i> , <i>Butyrivibrio</i> , & <i>Desulfovibrio</i> ; underrepresentation of <i>Turicibacter</i> & the <i>Lachnospiraceae</i> family
Hayashi et al., 2018	HF	Plasma/fecal	16 S rRNA gene amplicon sequencing/ CE-TOFMS	HF failure patients had higher plasma TMAO and IS levels than healthy subjects in HF & IS	Actinobacteria was enriched and <i>Bifidobacterium</i> was abundant, while <i>Megamonas</i> was depleted in HF patients compared to healthy subjects.
Li et al., 2018	GDM	Plasma	LC-MS/MS	Increase in plasma TMAO concentrations correlated with higher odds of GDM	Not investigated
F. Liu et al., 2017	T2DM	Blood/urine	16 S rRNA gene amplicon sequencing	Carbohydrate & amino acid metabolism was disturbed in T2DM patients	Increased Actinobacteria phylum; <i>Lactobacillus</i> abundance decreased with age & menopause; increased <i>Lactobacillus</i> correlated positively with FBG & UGLU. Decreased <i>Akkermansia muciniphila</i> was associated with FBG & UGLU.
R. Liu et al., 2017	Obesity	Plasma/fecal	Metagenomic sequencing/HPLC-MS	Obese individuals had increased serum concentration of 13 metabolites including glutamate, phenylalanine, tyrosine, leucine, isoleucine and valine compared to lean control subjects. Weight-loss intervention by bariatric surgery in 23 subjects who were obese at baseline associated with decreased serum glutamate concentration 3 months after surgery.	Marked decrease in <i>B.thetaiotaomicron</i> in obese individuals; weight-loss intervention by bariatric surgery associated with the microbiome in obese individuals become more similar to that in lean controls including increased abundance of <i>B. thetaiotaomicron</i> 3 month after surgery.
Liu et al., 2018	CAD	Plasma	RRLC-QTOF/MS	High plasma concentration of TMAO correlated with a thinner fibrous cap thickness, higher frequency of microvessels & increased incidence of thin-cap fibroatheroma	Not investigated
Matsuzawa et al., 2019	STEMI	Plasma	LC-MS/MS	Plasma TMAO levels significantly increased from the onset of STEMI event to 10 months after a myocardial infarction (chronic phase). Chronic-phase TMAO level independently predicted future cardiovascular events.	Not investigated
Missailidis et al., 2018	HIV-I	Plasma/fecal	16 S rRNA gene amplicon sequencing/ LC-MS/MS	TMAO levels were lower in untreated HIV-I-infected individuals & increased significantly after ART initiation	Bacteroidetes inversely correlated with TMAO levels, while Firmicutes positively correlated with TMAO levels.
Moon et al., 2018	DM & HIV or risk for HIV	Plasma/fecal	16 S rRNA gene amplicon sequencing/ LC-MS/MS	In diabetic women with or at high risk for HIV infection, plasma levels of several metabolites in tryptophan catabolism (e, g, kynurenine/tryptophan ratio), branched-chain amino acid & proline metabolism pathways were higher, while glycerophospholipids were lower.	Among women with or at high risk for HIV infection, DM was associated with decreased relative abundances of <i>Finnegoldia</i> , <i>Anaerococcus</i> , <i>Sneathia</i> , & <i>Adlercreutzia</i> genera.
Nie et al., 2018	Stroke	Serum	LC-MS/MS	Increase in TMAO levels significantly correlated with higher risk of first stroke	Not investigated

(continued)

Table 1. (continued)

Authors/Year	Disease/ Symptoms	Metabolite/ Microbial Samples	Analysis Method	Metabolite/Metabolic Pathways	Main Outcomes
Ortger et al., 2018	COPD	Plasma	LC-MS/MS	Increased TMAO levels were associated with long-term all-cause mortality in patients with COPD, but the association was not significant after controlling for comorbidities and age.	Not investigated
Ottosson et al., 2018	Obesity	Plasma/fecal	16 S rRNA gene amplicon sequencing/ LC-MS/MS	Glutamate & branched-chain amino acids strongly associated with elevated BMI	Blautia, Dorea, Ruminococcus, & SHA-98 genera were associated with BMI-predictive plasma metabolites, including glutamate & branched-chain amino acids.
Serena et al., 2018	Obesity	Plasma/serum/fecal	16 S rRNA gene amplicon sequencing/ LC-MS/MS	Obesity was associated with elevated levels of succinate & impaired glucose metabolism	Higher relative abundance of succinate-producing <i>Prevotellaceae</i> & <i>Veillonellaceae</i> , & a lower relative abundance of succinate-consuming <i>Odoribacteraceae</i> & <i>Clostridiaceae</i> in obese individuals
Shan et al., 2018	HIV and carotid atherosclerosis progression	Plasma	LC-MS/MS	TMAO was associated with an increased risk of carotid plaque in HIV-infected participants	Not investigated
Tuomainen et al., 2018	T2D	Serum	HPLC-QQQ-MS/MS	Higher IPA at 1-year study was inversely associated with the incidence of T2D. IPA levels positively correlated with dietary fiber intake & negatively with hsCRP concentration.	No investigated
Urpil-Sarda et al., 2019	T2D	Urine	HPLC-MS & IH-NMR spectroscopy	T2D was associated with high levels of methylsuccinate, alanine, dimethylglycine & guanidoacetate, & reduced levels of glutamine, methylguanidine, 3-hydroxymandelate & hippurate.	Not investigated
Walejko et al., 2018	Hypertension	Plasma/fecal	16 S rRNA gene amplicon sequencing/ UPLC	Black Americans with high blood pressure had significantly higher levels of sulfacetaldehyde, quinolinic acid, 5-aminolevulinic acid, leucine & phenylalanine & lower levels of 4-oxoproline & L-anserine.	Gut microbial taxonomy was different between Black Americans with high blood pressure & White Americans with high blood pressure. The microbiome of Black Americans with high blood pressure was enriched in functional pathways.
Wang et al., 2019	HIV	Plasma/fecal	16 S rRNA gene amplicon sequencing/ LC-MS/MS	HIV-infected women presented elevated glycerophospholipid levels compared to women without HIV infection.	HIV-infected women had higher abundances of <i>Ruminococcus</i> & <i>Oscillospira</i> & lower abundances of <i>Bifidobacterium</i> & <i>Collinsella</i> compared to women without HIV infection.
Zhai et al., 2019	Ischemic stroke	Plasma	LC-MS/MS	Patients with TMAO levels in the highest quartile were more likely to have higher risks of poor functional outcome & mortality	Not investigated
Zhu et al., 2020	PSCI	Plasma	LC-MS/MS	Higher level of plasma TMAO was an independent predictor for PSCI	Not investigated

Note. ¹H NMR = proton nuclear magnetic resonance; ART = antiretroviral; BMI = body mass index; CAD = coronary artery disease; CE-TOFMS = capillary electrophoresis time-of-flight mass spectrometry; COPD = chronic obstructive pulmonary disease; DM = diabetes mellitus; FBG = fasting blood glucose; GDM = gestational diabetes mellitus; HF = heart failure; HIV-1 = human immunodeficiency virus; HPLC-MS = high-performance liquid chromatography-mass spectrometry; HPLC-QQQ-MS/MS = liquid chromatography with mass spectrometric detection; hsCRP = high-sensitivity C-reactive protein; IPA = indolepropionic acid; IS = indoxyl sulfate; LC-MS/MS = liquid chromatography-tandem mass spectrometry; NMR = nuclear magnetic resonance; PSCI = Post-stroke cognitive impairment RRLC-QTOF/MS = rapid resolution liquid chromatography quadrupole time-of-flight mass spectrometry; STEMI = ST-segment elevation acute myocardial infarction; T2DM = Type 2 diabetes mellitus; TMAO = trimethylamine N-oxide; UGLU = urine glucose; UPLC = ultra-performance liquid chromatography.

R. Liu et al. (2017) reported that the abundance of *Bacteroides thetaiotaomicron* (phylum Bacteroidetes) was decreased in obese individuals relative to healthy controls and inversely correlated with serum levels of glutamate. In addition, Ottosson et al. (2018) found a strong positive association between plasma glutamate levels and body mass index. Serena et al. (2018) identified elevated plasma levels of succinate in obese subjects, and increased succinate was associated with a higher relative abundance of *Prevotellaceae* and *Veillonellaceae* (putative succinate-producing bacteria) and a lower relative abundance of *Odoribacteraceae* and *Clostridaceae* (putative succinate-consuming bacteria).

Researchers have used blood samples extensively to study the effects of chronic inflammatory and infectious processes on metabolic parameters. When Bellocchi et al. (2018) used HPLC to analyze plasma samples from healthy controls and patients with systemic sclerosis, they found peaks in glycerophospholipid metabolites in the patients with systemic sclerosis. The elevated glycerophospholipid metabolites were associated with an overrepresentation of pro-inflammatory gastrointestinal microbes, such as *Desulfovibrio*. This increase in putative pro-inflammatory microbial flora may adversely affect the integrity of the intestine, leading to the presence of metabolites in the circulation that activate inflammatory pathways.

Studies about chronic HIV infection have provided additional insights into the relationships among metabolic parameters, gut dysbiosis, antiretroviral treatment, and comorbidities. Moon et al. (2018), for example, found elevated levels of metabolites from tryptophan catabolism in the plasma of women with HIV. In a comparison of women with and without HIV infection, Wang et al. (2019) found that HIV was associated with elevated plasma glycerophospholipid levels, a structural element of the lipid component of cell membranes. In that study, bacteria from the genera *Ruminococcus* and *Oscillospira* were enriched and bacteria from the probiotic genus *Bifidobacterium* were depleted in HIV-infected women, suggesting an effect on glycerophospholipid metabolism. However, the potential functional consequences of these differences remain to be addressed in future studies.

Researchers have also used plasma TMAO to assess cardiovascular disease risk in individuals with HIV. Shan et al. (2018) found that plasma TMAO levels predicted an increased risk for carotid artery plaque, but the relationship was attenuated ($p = 0.08$) after they controlled for circulating markers of monocyte activation and inflammation. In another study, authors challenged the notion that TMAO may be a reliable marker for cardiovascular risk in patients with HIV who undergo long-standing antiretroviral treatment. Missailidis et al. (2018) found that elevated plasma TMAO levels did not predict cardiovascular risk in these cases; rather TMAO levels were positively correlated with Firmicutes and inversely correlated with Bacteroidetes in this study of subjects receiving antiretroviral drugs. The researchers concluded that TMAO may not be a useful biomarker of cardiovascular health in patients taking antiretrovirals because the treatment may alter the microbial composition of the gastrointestinal system.

Urine Testing

Three studies included urinary metabolomic and microbiome measures. These studies focused on defining urinary metabolism profiles to correlate with disease presentation. Urpi-Sarda et al. (2019) found that high blood glucose levels were associated with elevated urinary phenylalanine, phenylacetylglutamine, p-cresol, and acetoacetate levels in patients with T2D. Data from Amin et al. (2019) suggested an enrichment of the methylhistidine metabolism pathway and perturbations in galactose and histidine metabolism in patients with coronary artery disease. In a study of the urinary microbiome of female patients with T2D, F. Liu et al. (2017) observed that an increased abundance of organisms from the Actinobacteria phylum was associated with higher blood (fasting) and urine glucose levels and body mass index. The urinary tract microbiome is generally dominated by bacteria of the genera *Lactobacillus*, *Gardnerella*, and *Prevotella*. F. Liu et al. (2017) observed that the abundance of *Lactobacillus* was positively correlated with blood and urine glucose levels and that *Lactobacillus* abundance also decreased with age and with menopause. Measurements of microbial diversity (e.g., bacterial richness) were reduced in individuals with T2D, and these metrics were associated with evidence of reduced carbohydrate and amino acid metabolism.

Sample Preparation Considerations

Standardization in sample collection, processing, and storage procedures is critical for reliable and valid results (Table 2). Investigators should carefully select blood collection tubes based on the type of metabolomics analyses. Some metabolites (short-chain fatty acids [SCFAs]) are much more volatile than others so it is critical know the characteristics of the metabolites of interest for developing sample-collection and -storage procedures. Tubes containing ethylenediaminetetraacetic acid (EDTA), for example, can cause mass spectrometry signal interference. For this reason, heparinized tubes are commonly used rather than tubes containing EDTA for plasma collection (Chen et al., 2019). In addition, the timing between blood collection and centrifugation can affect results. If a delay in centrifuging blood is inevitable, Jobard et al. (2016) recommend storing blood at 4°C to improve stability of the metabolites. To avoid the gradual degradation of metabolites, centrifuge, aliquot, and store plasma and serum samples at -80°C .

For urine, midstream collection is recommended, and the sample should undergo centrifugation (1000 to 3000 g for 5 min at 4°C) to remove pellets and cells before long-term storage (Chen et al., 2019). It is important to consider that the microbiomes of voided and catheterized urine will differ substantially: voided urine will contain microbes from the genitourinary tract, which may be of interest or not, depending on the aims of the study (Wolf & Brubaker, 2019). Before quantifying urinary metabolites, investigators may need to perform concentration normalization procedures based on creatinine and osmolality values (Chen et al., 2019).

Table 2. Recommendations for Sample Preparation.

Blood Specimens	Urine Specimens
Select the appropriate additives (e.g., heparin) for isolating plasma. The methods will depend on the specific analytic method.	Standardize the urine-collection procedures. If the study will examine the urinary microbiota, be aware that voided versus catheterized urine will have substantially different profiles.
Store blood at -4°C if samples cannot be centrifuged immediately after collection.	For voided samples, obtain midstream urine to reduce bacterial contamination from the skin & genitourinary tract.
	Use normalization procedures based on creatinine & osmolality values, as directed for the specific analytic method.
Separate the collected sample into aliquots to allow for repeat testing without the sample undergoing freeze–thaw cycles. Store at low temperature for metabolite stability (-80°C).	
Include fecal sampling at each specimen-collection timepoint when possible to allow metabolic results to be correlated with changes in fecal bacterial characteristics.	
Standardize subjects' diets when possible to reduce within- & between-subject variation; collect detailed food-intake diaries & measure relevant covariates.	

Both circulating and excreted concentrations of metabolites are affected by a variety of covariates (e.g., dietary intake, medications, hormone/contraceptive use, sex, race, age, and menopausal status). The values of biomarkers related to these covariates are important considerations for study design. TMAO levels, for example, are affected by diet, liver-enzyme activity, and the rate of methylamines excretion (Nowinski & Ufnal, 2018). Thus, Nie et al. (2018) adjusted for serum levels of the TMAO precursors (choline, carnitine) in the final analyses. These considerations emphasize the importance of collecting samples to quantify all relevant covariates that will be needed to interpret study results.

Discussion

To our knowledge this review is the first to examine recent literature about blood and urine metabolomics, especially as the findings and methods relate to the advancement of nursing science. The following discoveries represent the main findings of this review. (1) TMAO levels may be predictive of cardiovascular outcomes in some cohorts; however, levels could be affected by medications that alter the composition of intestinal bacteria. (2) Metabolites of tryptophan catabolism (e.g., IPA) could provide insight into endocrine and inflammatory pathways, and changes in the relative abundances of bacteria in the colon or urinary tract could have profound effects on multiple metabolic pathways. (3) In addition, gender- and race-related differences in microbiome and metabolic parameters have been understudied. The literature suggests that important differences exist, which, if understood, could be used to design more

tailored health-promotion strategies. The reviewed studies were exploratory; until more knowledge develops it is important for researchers to quantify the fecal, blood, and urine levels of the variables of interest in future studies.

The recent discoveries highlight important opportunities for future nursing studies. In general, nurse researchers conducted studies to define symptoms, develop disease-prevention strategies, and design interventions that empower patients to cope with health conditions. By identifying alterations in metabolic pathways, for example, researchers could aim to understand pathophysiologic processes underlying hyperglycemia/hypoglycemia symptoms and then develop interventions targeting the associated pathways (e.g., design a nutritional program that improves outcomes with T2D). Nursing initiatives for cardiovascular-disease prevention could also address specific needs of different populations after researcher defined how demographic characteristics affect the composition of the microbiome and the metabolic pathways contributing to cardiovascular disease.

Historically, research conducted within the nursing profession has focused on understanding environment–health interactions. During the Crimean War, nurses led by Florence Nightingale documented the prevalence of battlefield deaths caused by gastrointestinal infections; mortality rates improved after Nightingale implemented initiatives to improve sanitation (Fee & Garofalo, 2010). The gastrointestinal microbiome and microbes in general had yet to be discovered at that time, but Nightingale and others' work during the Crimean War nevertheless set a foundation for nurses' future roles in infection control and disease prevention. The present review illustrates how the recent advances in metabolomics provide opportunities for nurses to be leaders in driving innovations that define the holistic effects of dysregulated gastrointestinal health. Similar to Nightingale's paradigm-shifting initiatives for improving sanitation, researchers can now use the existing and emerging metabolomics and microbiome technologies to address nursing-focused research questions.

In summary, nurses' research about disease prevention and symptom science, as well as studies testing the impact of nursing interventions, could be strengthened by including blood and urine markers related to the microbiome. As the nursing profession moves forward in this era of precision health, the integration of metabolomic technologies into nursing research will lead to the discovery of microbiome-based biomarkers and microbiome-targeted therapies for optimizing health as well as for preventing, detecting, and managing disease.

Abbreviations

EDTA	Ethylenediaminetetraacetic acid
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
IPA	Indolepropionic acid
NMR	Nuclear magnetic resonance
T2D	Type 2 diabetes
TMAO	Trimethylamine N-oxide

Authors' Note

The opinions expressed herein and the interpretation and reporting of these data are the responsibility of the author(s) and should not be seen as an official recommendation or interpretation of the National Institutes of Health. The review process for this manuscript was handled by guest editor Dr. Michelle Wright and editor-in-chief Dr. Carolyn Yucha, as one of the authors, Dr. Paule Joseph, is also a guest editor of this special issue.

Acknowledgments

The authors would like to thank Brigit Sullivan, librarian at the National Institutes of Health (NIH) Library, for reference assistance and Dr. Joan Austin and Melissa Cisewski for their comments in this paper.

AFD, PVJ and WAH are forever grateful to Dr. Nicolaas H. Fourie for his mentoring and commitment and contribution to the advancement of nursing science in microbiome research.

Author Contributions

All authors contributed equally to the conception and design of the study, acquisition of data, and manuscript drafting and critical revision.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: PVJ is supported by the National Institute of Nursing Research under award number 1Z1ANR000035-01. PVJ is also supported by the Office of Workforce Diversity, National Institutes of Health and the Rockefeller University Heilbrunn Nurse Scholar Award. AR and ATF received Postbaccalaureate Intramural Research Training Awards, Office of Intramural Training & Education, National Institutes of Health, Department of Health and Human Services. KAM received support from Sigma Theta Tau International Alpha Lambda Chamber, the University of Illinois at Chicago and the International Society of Nurses in Genetics.

ORCID iDs

Ana F. Diallo <https://orcid.org/0000-0003-1547-2062>
 Katherine A. Maki <https://orcid.org/0000-0003-4578-960X>
 Alexis T. Franks <https://orcid.org/0000-0002-0550-334X>
 Rosario Jaime-Lara <https://orcid.org/0000-0002-5726-7529>
 Paule V. Joseph <https://orcid.org/0000-0002-1198-9622>
 Wendy A. Henderson <https://orcid.org/0000-0003-3924-7118>
 Stefan J. Green <https://orcid.org/0000-0003-2781-359X>

References

- Amin, A. M., Mostafa, H., Arif, N. H., Abdul Kader, M. A. S., & Kah Hay, Y. (2019). Metabolomics profiling and pathway analysis of human plasma and urine reveal further insights into the multifactorial nature of coronary artery disease. *Clinica Chimica Acta*, 493, 112–122. <https://doi.org/10.1016/j.cca.2019.02.030>
- Anesi, A., Rubert, J., Oluwagbemigun, K., Orozco-Ruiz, X., Nöthlings, U., Breteler, M. M. B., & Mattivi, F. (2019). Metabolic profiling of human plasma and urine, targeting tryptophan, tyrosine and branched chain amino acid pathways. *Metabolites*, 9, E261. <https://doi.org/10.3390/metabo9110261>
- Baxter, N. T., Schmidt, A. W., Venkataraman, A., Kim, K. S., Waldron, C., & Schmidt, T. M. (2019). Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers. *MBio*, 10, e02566–18. <https://doi.org/10.1128/mBio.02566-18>
- Bellocchi, C., Fernández-Ochoa, Á., Montanelli, G., Vigone, B., Santaniello, A., Milani, C., Quirantes-Piné, R., Borrás-Linares, I., Ventura, M., Segura-Carrettero, A., Alarcón-Riquelme, M. E., & Beretta, L. (2018). Microbial and metabolic multi-omic correlations in systemic sclerosis patients. *Annals of the New York Academy of Sciences*, 1421, 97–109. <https://doi.org/10.1111/nyas.13736>
- Bugaut, M. (1987). Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 86(3), 439–472.
- Chen, M. X., Wang, S. Y., Kuo, C. H., & Tsai, I. L. (2019). Metabolome analysis for investigating host-gut microbiota interactions. *Journal of the Formosan Medical Association*, 118, S10–S22. <https://doi.org/10.1016/j.jfma.2018.09.007>
- Chhibber-Goel, J., Singhal, V., Parakh, N., Bhargava, B., & Sharma, A. (2017). The metabolite trimethylamine-N-oxide is an emergent biomarker of human health. *Current Medicinal Chemistry*, 24, 3942–3953. <https://doi.org/10.2174/0929867323666160830104025>
- Connors, J., Dawe, N., & Van Limbergen, J. (2018). The role of succinate in the regulation of intestinal inflammation. *Nutrients*, 11, E25. <https://doi.org/10.3390/nu11010025>
- Daliri, E. B., Wei, S., Oh, D. H., & Lee, B. H. (2017). The human microbiome and metabolomics: Current concepts and applications. *Critical Reviews in Food Science and Nutrition*, 57, 3565–3576. <https://doi.org/10.1080/10408398.2016.1220913>
- Fee, E., & Garofalo, M. E. (2010). Florence nightingale and the Crimean war. *American Journal of Public Health*, 100, 1591. <https://doi.org/10.2105/AJPH.2009.188607>
- Hayashi, T., Yamashita, T., Watanabe, H., Kami, K., Yoshida, N., Tabata, T., Emoto, T., Sasaki, N., Mizoguchi, T., Irino, Y., Toh, R., Shinohara, M., Okada, Y., Ogawa, W., Yamada, T., & Hirata, K. I. (2018). Gut microbiome and plasma microbiome-related metabolites in patients with decompensated and compensated heart failure. *Circulation Journal*, 83, 182–192. <https://doi.org/10.1253/circj.CJ-18-0468>
- Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J. H., Chinwalla, A. T., Creasy, H. H., Earl, A. M., Fitzgerald, M. G., Fulton, R. S., Giglio, M. G., Hallsworth-Pepin, K., Lobos, E. A., Madupu, R., Magrini, V., Martin, J. C., Mitreva, M., Muzny, D. M., Sodergren, E. J., . . . White, O. (2012). Structure, function and diversity of the healthy human microbiome. *Nature*, 486, 207–214. <https://doi.org/10.1038/nature11234>
- Jobard, E., Tredan, O., Postoly, D., Andre, F., Martin, A. L., Elena-Herrmann, B., & Boyault, S. (2016). A systematic evaluation of blood serum and plasma pre-analytics for metabolomics cohort studies. *International Journal of Molecular Sciences*, 17, E2035. <https://doi.org/10.3390/ijms17122035>
- Li, P., Zhong, C., Li, S., Sun, T., Huang, H., Chen, X., Zhu, Y., Hu, X., Peng, X., Zhang, X., Bao, W., Shan, Z., Cheng, J., Hu, F. B., Yang, N., & Liu, L. (2018). Plasma concentration of trimethylamine-N-oxide and risk of gestational diabetes mellitus. *American Journal of Clinical Nutrition*, 108, 603–610. <https://doi.org/10.1093/ajcn/nqy116>

- Liu, F., Ling, Z., Xiao, Y., Xiao, Y., Lv, L., Yang, Q., Wang, B., Lu, H., Zheng, L., Jiang, P., Wang, W., & Li, L. (2017). Dysbiosis of urinary microbiota is positively correlated with type 2 diabetes mellitus. *Oncotarget*, 8, 3798–3810. <https://doi.org/10.18632/oncotarget.14028>
- Liu, R., Hong, J., Xu, X., Feng, Q., Zhang, D., Gu, Y., Shi, J., Zhao, S., Liu, W., Wang, X., Xia, H., Liu, Z., Cui, B., Liang, P., Xi, L., Jin, J., Ying, X., Wang, X., Zhao, X., ... Wang, W. (2017). Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nature Medicine*, 23, 859–868. <https://doi.org/10.1038/nm.4358>
- Liu, X., Xie, Z., Sun, M., Wang, X., Li, J., Cui, J., Zhang, F., Yin, L., Huang, D., Hou, J., Tian, J., & Yu, B. (2018). Plasma trimethylamine N-oxide is associated with vulnerable plaque characteristics in CAD patients as assessed by optical coherence tomography. *International Journal of Cardiology*, 265, 18–23. <https://doi.org/10.1016/j.ijcard.2018.04.126>
- Maki, K. A., Diallo, A. F., Lockwood, M. B., Franks, A. T., Green, S. J., & Joseph, P. V. (2018). Considerations when designing a microbiome study: Implications for nursing science. *Biological Research for Nursing*, 21, 125–141. <https://doi.org/10.1177/1099800418811639>
- Matsuzawa, Y., Nakahashi, H., Konishi, M., Sato, R., Kawashima, C., Kikuchi, S., Akiyama, E., Iwahashi, N., Maejima, N., Okada, K., Ebina, T., Hibi, K., Kosuge, M., Ishigami, T., Tamura, K., & Kimura, K. (2019). Microbiota-derived trimethylamine N-oxide predicts cardiovascular risk after STEMI. *Scientific Reports*, 9, 11647. <https://doi.org/10.1038/s41598-019-48246-6>
- Missailidis, C., Neogi, U., Stenvinkel, P., Trøseid, M., Nowak, P., & Bergman, P. (2018). The microbial metabolite trimethylamine-N-oxide in association with inflammation and microbial dysregulation in three HIV cohorts at various disease stages. *AIDS*, 32, 1589–1598. <https://doi.org/10.1097/QAD.0000000000001813>
- Moon, J. Y., Zolnik, C. P., & Wang, Z. (2018). Gut microbiota and plasma metabolites associated with diabetes in women with, or at high risk for, HIV infection. *EBioMedicine*, 37, 392–400.
- Nie, J., Xie, L., Zhao, B. X., Li, Y., Qiu, B., Zhu, F., Li, G. F., He, M., Wang, Y., Wang, B., Liu, S., Zhang, H., Guo, H., Cai, Y., Huo, Y., Hou, F. F., Xu, X., & Qin, X. (2018). Serum trimethylamine N-oxide concentration is positively associated with first stroke in hypertensive patients. *Stroke*, 49, 2021–2028. <https://doi.org/10.1161/STROKEAHA>
- Nowiński, A., & Ufnal, M. (2018). Trimethylamine N-oxide: A harmful, protective or diagnostic marker in lifestyle diseases? *Nutrition*, 46, 7–12. <https://doi.org/10.1016/j.nut.2017.08.001>
- Ottiger, M., Nickler, M., Steuer, C., Bernasconi, L., Huber, A., Christ-Crain, M., Henzen, C., Hoess, C., Thomann, R., Zimmerli, W., Mueller, B., & Schuetz, P. (2018). Gut, microbiota-dependent trimethylamine-N-oxide is associated with long-term all-cause mortality in patients with exacerbated chronic obstructive pulmonary disease. *Nutrition*, 45, 135–141. <https://doi.org/10.1016/j.nut.2017.07.001>
- Ottosson, F., Brunkwall, L., Ericson, U., Nilsson, P. M., Almgren, P., Fernandez, C., Melander, O., & Orho-Melander, M. (2018). Connection between BMI-related plasma metabolite profile and gut microbiota. *Journal of Clinical Endocrinology and Metabolism*, 103, 1491–1501. <https://doi.org/10.1210/jc.2017-02114>
- Pavlova, T., Vidova, V., Bienertova-Vasku, J., Janku, P., Almasi, M., Klanova, J., & Spacil, Z. (2017). Urinary intermediates of tryptophan as indicators of the gut microbial metabolism. *Analytica Chimica Acta*, 987, 72–80. <https://doi.org/10.1016/j.aca.2017.08.022>
- Serena, C., Ceperuelo-Mallafré, V., Keiran, N., Queipo-Ortuño, M. I., Bernal, R., Gomez-Huelgas, R., Urpi-Sarda, M., Sabater, M., Pérez-Brocá, V., Andrés-Lacueva, C., Moya, A., Tinahones, F. J., Fernández-Real, J. M., Vendrell, J., & Fernández-Veledo, S. (2018). Elevated circulating levels of succinate in human obesity are linked to specific gut microbiota. *Multidisciplinary Journal of Microbial Ecology*, 12, 1642–1657. <https://doi.org/10.1038/s41396-018-0068-2>
- Shan, Z., Clish, C. B., Hua, S., Scott, J. M., Hanna, D. B., Burk, R. D., Haberman, S. A., Shah, S. J., Margolick, J. B., Sears, C. L., Post, W. S., Landay, A. L., Lazar, J. M., Hodis, H. N., Anastos, K., Kaplan, R. C., & Qi, Q. (2018). Gut microbial-related choline metabolite trimethylamine-N-oxide is associated with progression of carotid artery atherosclerosis in HIV infection. *Journal of Infectious Diseases*, 218, 1474–1479. <https://doi.org/10.1093/infdis/jiy356>
- Tuomainen, M., Lindström, J., Lehtonen, M., Auriola, S., Pihlajamäki, J., Peltonen, M., Tuomilehto, J., Uusitupa, M., de Mello, V. D., & Hanhineva, K. (2018). Associations of serum indolepropionic acid, a gut microbiota metabolite, with type 2 diabetes and low-grade inflammation in high-risk individuals. *Nutrition & Diabetes*, 8, 35. <https://doi.org/10.1038/s41387-018-0046-9>
- Urpi-Sarda, M., Almanza-Aguilera, E., Llorach, R., Vázquez-Fresno, R., Estruch, R., Corella, D., Sorli, J. V., Carmona, F., Sanchez-Pla, A., Salas-Salvadó, J., & Andres-Lacueva, C. (2019). Non-targeted metabolomic biomarkers and metabolites of type 2 diabetes: A cross-sectional study of PREDIMED trial participants. *Diabetes & Metabolism*, 45, 167–174. <https://doi.org/10.1016/j.diabet.2018.02.006>
- Velasquez, M. T., Ramezani, A., Manal, A., & Raj, D. S. (2016). Trimethylamine N-oxide: The good, the bad and the unknown. *Toxins*, 8, E326. <https://doi.org/10.3390/toxins8110326>
- Walejko, J. M., Kim, S., Goel, R., Handberg, E. M., Richards, E. M., Pepine, C. J., & Raizada, M. K. (2018). Gut microbiota and serum metabolite differences in African Americans and White Americans with high blood pressure. *International Journal of Cardiology*, 271, 336–339. <https://doi.org/10.1016/j.ijcard.2018.04.074>
- Wang, Z., Usyk, M., & Sollecito, C. C. (2019). Altered gut microbiota and host metabolite profiles in HIV-infected women. *Clinical Infectious Diseases*, ciz1117. <https://doi.org/10.1093/cid/ciz1117>
- Wolfe, A. J., & Brubaker, L. (2019). Urobiome updates: Advances in urinary microbiome research. *Nature Reviews Urology*, 16, 73–74. <https://doi.org/10.1038/s41585-018-0127-5>
- Zhai, Q., Wang, X., Chen, C., Tang, Y., Wang, Y., Tian, J., Zhao, Y., & Liu, X. (2019). Prognostic value of plasma trimethylamine N-oxide levels in patients with acute ischemic stroke. *Cellular and Molecular Neurobiology*, 39, 1201–1206. <https://doi.org/10.1007/s10571-019-00714-3>
- Zhu, C., Li, G., & Lv, Z. (2020). Association of plasma trimethylamine-N-oxide levels with post-stroke cognitive impairment: A 1-year longitudinal study. *Neurological Sciences*, 41, 57–63. <https://doi.org/10.1007/s10072-019-04040-w>