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Human Suction Blister Fluid Composition Determined Using High-Resolution Metabolomics

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ABSTRACT: Interstitial fluid (ISF) surrounds the cells and tissues of the body. Since ISF has molecular components similar to plasma, as well as compounds produced locally in tissues, it may be a valuable source of biomarkers for diagnostics and monitoring. However, there has not been a comprehensive study to determine the metabolite composition of ISF and to compare it to plasma. In this study, the metabolome of suction blister fluid (SBF), which largely consists of ISF, was analyzed using untargeted high-resolution metabolomics (HRM). A wide range of metabolites were detected in SBF, including amino acids, lipids, nucleotides, and compounds of exogenous origin. Various systemic and skin-derived metabolite biomarkers were elevated or found uniquely in SBF, and many other metabolites of clinical and physiological significance were well correlated between SBF and plasma. In sum, using untargeted HRM profiling, this study shows that SBF can be a valuable source of information about metabolites relevant to human health.

Biomarkers are a powerful tool to study the entire spectrum of disease, from the ascertainment of diagnosis, examination of disease progression, and assessment of therapeutic benefits.¹ Most commonly, biomarkers are measured in easily accessible body fluids, such as blood, salvia, and urine. However, there are challenges associated with these fluids. Blood sampling by venipuncture is invasive, potentially painful, and requires trained personnel. Biomarker concentrations in blood samples obtained via finger pricks may be variable if not collected under properly controlled conditions.² Many saliva biomarkers are detected at low concentrations, and there is potential for interference by food or drugs.³ While urine samples are easier to obtain than blood, urinary biomarker concentrations can be highly variable due to differences in urine dilution.⁴

Although interstitial fluid (ISF) constitutes 60% of total body fluids in humans,⁵ it is greatly unexplored as a matrix for biomarker detection. ISF, which bathes and surrounds cells and tissues of the body, is formed as plasma traverses blood vessels and equilibrates with the cell and tissue environment (reviewed in refs 6). ISF provides a means of delivering nutrients to cells, enables intercellular communication, and removes metabolic waste.

The detection of biomarkers in ISF has several advantages to blood, urine, and saliva. Since ISF interacts directly with intracellular fluid, it is possible that compounds that cannot be detected in plasma may be detected in ISF. Unlike blood, ISF can be used for continuous biomarker monitoring, in part because it does not clot.⁷ For example, continuous glucose monitors sample ISF to measure glucose concentrations.⁸ Unlike plasma, which provides an integrated measurement of biomarkers from multiple organ and metabolic systems, ISF can capture changes in the local environment.⁹ For instance, ISF sampled from tumors has been studied as a source of cancer biomarkers.¹⁰,¹¹ In addition, ISF has lower concentrations of high-abundance proteins like albumin and globulin compared to plasma,¹² which makes it easier to screen ISF for low-abundance compounds without extensive protein depletion and sample cleanup strategies.

There are limited techniques for ISF sampling, including suction blisters,¹³ microdialysis,¹⁴ open flow microperfusion,¹⁵ reverse iontophoresis,¹⁶ and microneedle patches.¹⁷,¹⁸ Suction blisters are a common method to sample large volumes of ISF, which are generated by applying vacuum to a skin area. A suction application separates the dermis and epidermis, and...
fluid from the surrounding tissues fills the gap, creating a blister. The blister fluid is withdrawn by a conventional needle and syringe. This fluid, referred to as SBF, is largely derived from ISF but may also contain some intracellular components and inflammatory markers that are a consequence of the collection method. Compared to other ISF sampling methods—

including reverse iontophoresis, wherein an electric current is applied to skin, or microdialysis, wherein a small membrane is inserted into skin—suction blister sampling is a relatively noninvasive method for ISF collection, since the method requires a single needle puncture, similar to venipuncture.

The protein composition of SBF sampled from suction blisters has been studied using mass spectrometry. From suction blisters obtained from eight healthy individuals, Muller et al. found that the proteome of SBF from suction blisters was heterogeneous, consisting of systemic plasma components, proteins originated from cell leakage, and proteins associated with skin tissue. Comparing proteins measured in SBF and plasma from six individuals, Kool et al. found that 83% of proteins found in plasma were also found in SBF, whereas only half of SBF proteins were common to plasma. The authors constructed a list of 34 clinically relevant protein biomarkers that were abundant in SBF: while 9 out of the 34 were epidermal-derived, the rest were known systemic biomarkers, suggesting the utility of SBF as a surrogate for blood-based biomarker detection, as well as a source of tissue-specific biomarkers.

While previous research has explored the protein composition of SBF, to date, no study has characterized the small-molecule composition of SBF. Metabolites provide critical information on metabolic pathway intermediates, disease states, and exposure to environmental agents. Metabolite profiling is also a key tool to study the exposome, an emerging research paradigm involving the investigation of complex environmental exposures, biological responses to these exposures, and their impacts on human health and disease.

Here, we profiled small-molecule metabolites in SBF obtained from suction blisters to better understand the composition of the SBF metabolome. First, metabolites in plasma and SBF samples obtained from human volunteers were analyzed using untargeted high-resolution metabolomics (HRM), which was used to characterize metabolites present in SBF. Second, we performed an untargeted screen for SBF metabolites that may be useful as biomarkers by identifying metabolite features that were elevated in SBF and/or strongly correlated between SBF and plasma. Collectively, the work characterizes the differences between the SBF and plasma metabolomes, as well as the potential utility of alternative biofluids for biomarker detection in clinical and exposome research.

## RESULTS

**Metabolomic Profiles of SBF and Plasma.** To examine the SBF and plasma metabolomes in volumes that can be feasibly sampled in clinical settings, we compared metabolite features detected in the volume of SBF collected via suction blister sampling (15 μL of SBF diluted to final volume of 50 μL with LC/MS grade H2O) against 50 μL of plasma collected via venipuncture, which is the standard sample volume used for this metabolomics assay. HRM detected 7044 m/z features that were present in SBF and/or plasma (Figure 1). Note that m/z features do not necessarily correspond one-to-one with chemical compounds and may represent multiple adducts, isotopes, or fragments from the same parent ion. Comparison of SBF to plasma showed that the large majority of features were detected in both biofluids, even though SBF had been diluted to provide the minimum volume required for HRM analysis. There were 1032 and 429 features unique to plasma and SBF, respectively, where “uniqueness” was defined as the detection of the feature in at least 1 of the 10 samples in one fluid but not in any of the 10 samples of the other biofluid.

We identified 105 metabolites (i.e., groups of adducts/isotopes derived from the same parent ion) with high confidence in the SBF samples that were matched to compounds in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database by accurate mass (see Supporting Information (SI)). We also identified metabolites by comparing features to a library of metabolite biomarkers relevant to clinical and exposome research; compound identities were obtained by matching the accurate mass m/z values and retention times of the features to analytical standards in the library. The final curated list of metabolites is presented in Table S1.

We detected a wide range of metabolites in SBF involved in amino acid metabolism, lipid metabolism, and nucleotide metabolism, as well as clinical biomarkers such as glucose, cholesterol, creatinine, and urea (Figure 2 and Table S1). Most endogenous metabolites were detected in the majority of SBF samples and were present in both SBF and plasma. We found matches for environmental toxicants, including several pesticides. Environmental compounds were generally detected in a greater number of plasma samples compared to SBF.

A limited number of metabolites were unique to plasma (Table S2). Several were environmental chemicals, including the pesticides malathion, nabam, and triadimefon; ammeline, a byproduct of the industrial compound melamine; and the mycotoxin aflatoxin. Two metabolites were artifacts of the venipuncture sampling: skin disinfection was carried out with an iodine-containing compound, and ethylenediaminetetraacetic acid (EDTA) tubes were used for blood collection.

**Metabolites Markedly Elevated in SBF.** To identify unique characteristics of the SBF metabolome, we identified features that were elevated in SBF using a paired fold-change analysis. Since the SBF samples were diluted, these metabolites should reflect compounds that are greatly elevated in SBF relative to plasma. A curated list of all unique and elevated features with putative compound matches in the Human Metabolomics Database (HMDB) is presented in Table 1.

While 316 features were unique to and elevated in SBF based on our criteria, only 23 compounds were identified by accurate mass m/z matching (Table 1). This may be because some of the molecules present in SBF have not yet been identified or are not included in the HMDB database. We manually classified these metabolites into several groups, including five phospholipids, three purines, two spermidines, two methionine-related compounds, six other endogenous compounds, and five dietary-derived compounds. Several of these metabolites are promising...
The large majority of endogenous metabolites in Tables S1 and S2 were detected in both SBF and plasma, confirming that common endogenous compounds can reliably be detected in SBF. Amino acid, lipid, and nucleotide metabolites, along with the clinical biomarkers cholesterol, glucose, creatinine, and urea, were detected in almost all of the SBF samples. Among the few metabolites that were not detected in SBF, many were environmental in origin, including several toxicants of potential relevance to environmental health. Considering the small sample volume of SBF used in the current study, it is possible that these metabolites were present in SBF but too low for reliable detection using this assay.

### DISCUSSION

This study describes the first detailed analysis of the metabolite composition of human SBF using untargeted HRM analysis. Matched SBF and plasma samples were collected from ten human volunteers, and clinically relevant sample volumes were analyzed by liquid chromatography with high-resolution mass spectrometry to find similarities and differences between the chemical compositions of the two biofluids. Although the majority of metabolite features were detected in both SBF and plasma, the biofluids had distinct characteristics. A diverse range of metabolites of endogenous and environmental origin were detected in SBF. Several glycerophospholipid-, purine-, and spermidine-associated metabolites were elevated in SBF, and many amino acids, nucleic acids, hormones, and exogenous compounds were well correlated between SBF and plasma. Altogether, our results suggest that metabolomic profiling of SBF has the potential to provide information about local and systemic biological activities and may be useful for monitoring established and novel biomarkers. In some cases, metabolite detection in SBF is a reliable proxy for blood, and in other cases, SBF contains metabolites that are absent from, or found in lower abundance, in blood.

To explore the biological significance of the positively correlated metabolites, we input the results into Mummichog for pathway and module analysis (Figure 3B). Metabolites correlated between plasma and SBF were commonly found in amino- acid-related pathways (e.g., urea cycle/amino group metabolism; glycine, serine, alanine, and threonine metabolism; aspartate and asparagine metabolism; Figure 3A). To explore the biological functions of the metabolites identified in the module analysis, KEGG IDs from the significant modules were input into KEGG BRITE, which found numerous peptides, lipids, and hormones/neurotransmitters among the correlated features (Figure 3B). The activity network, which contains metabolites whose identities could be predicted with high confidence, contained several amino acids (e.g., proline, glycine, homocysteine, betaine, methionine, tyrosine), nucleic acids (e.g., guanosine, guanine, uracil), and neurotransmitter-related metabolites (e.g., dopamine, methylhistamine; Figure S1)).

We compared these results against metabolites identified in a manual annotation of highly correlated features. Here, we manually annotated 99 features with strong correlations between SBF and plasma (Spearman rho > 0.7) using HMDB (Table 2). Many metabolites were identified both in the activity network and Table 2, including homocysteine, betaine, methionine, proline, tyrosine, acetylcarnitine, and octenoylcarnitine, strengthening the evidence for these compound identifications. Results from pathway-associated metabolite set enrichment analysis (MSEA) for metabolites in Table 2 found over-representation of metabolites in protein biosynthesis, betaine metabolism, methionine metabolism, and glycine, serine, and threonine metabolism (p < 0.05), consistent with Mummichog pathway results. Metabolites annotated manually that were not identified by Mummichog were trimethylamine N-oxide (TMAO), a microbiota-dependent compound linking carnitine and betaine metabolism that has been identified as a promising biomarker of cardiovascular disease, and caffeine and trigonelline, two compounds related to coffee consumption.33

![Figure 2. Types of metabolites detected in SBF. Figure displays the classes of metabolites identified in SBF. Bars reflect the number of metabolites detected for each class, with endogenous and environmental compounds denoted by green and purple bars, respectively. The full list of individual metabolites can be found in Table S1.](image-url)
A primary goal of our study was to examine the usefulness of SBF sampling for metabolite biomarker detection as a substitute or companion to blood sampling via venipuncture. We identified several metabolites that were elevated in SBF compared to plasma, which reflect metabolites that may be uniquely and/or more easily assessed in SBF. Urocanic acid, an epidermal metabolite that accumulates in the stratum corneum, was unique to SBF. While it is a chromophore for ultraviolet radiation acting as a "natural sunscreen," it has also been reported to have immunosuppressive effects and may play a detrimental role in photocarcinogenesis. Spermidine, which has also been reported to be elevated in the epidermis, is a polyamine compound associated with angiogenic mechanisms. Spermidine has promise as a biomarker for cancer aggressiveness, suicidal behavior in mood disorders, and response to breast cancer therapies. Phosphocreatinine and creatine are important components of energy metabolism, and creatine has been identified as a potential biomarker of mitochondrial diseases. Hypoxanthine and inosine, nucleotide bases formed during purine metabolism, are increased in response to injury and are biomarkers for cardiac ischemia; however, future work should examine the impact of the suction blister sampling protocol on the levels of these metabolites.

Several phospholipid-related compounds were elevated in SBF. Glycerophosphocholine, a building block for phospholipids in cell membranes, is a biomarker for breast cancer and myeloma, as well as Alzheimer's disease. Glycophosphocholine and glycercylophosphorylethanolamine in semen have been implicated as biomarkers for infertility problems. O-Phosphoethanolamine has promise as a biomarker for major depressive disorder and amyotrophic lateral sclerosis. Exogenous food-based compounds were also elevated in SBF. One compound unique to SBF, 3-methylsulfinylpropyl isothiocyanate, is obtained through the consumption of cruciferous vegetables. Isothiocyanates, which are phytochemicals with chemoprotective activities, are believed to contribute to the health benefits of vegetable-rich diets. Recently, a screen for toll-like receptor (TLR) inhibitors in vegetable extracts identified 3-methylsulfinylpropyl isothiocyanate as a compound with potent anti-inflammatory effects. Another food-derived compound unique to SBF, 2,3,4-trimethyltリアcan- tane, is found in fruits. While preliminary, our findings highlight the possibility for SBF as a unique source of dietary biomarkers.

We also identified metabolites that were positively correlated between SBF and plasma, which reflect metabolites whose SBF levels are informative of their blood levels. Many amino acids, neurotransmitters, and nucleic acids were strongly correlated between the fluids. Several clinically relevant metabolites were also strongly correlated between SBF and plasma. Elevated homocysteine, a sensitive indicator of B-vitamin deficiency, is a biomarker for cardiovascular and neurodegenerative conditions. Creatinine is an important biomarker for kidney function. Betaine is a metabolite that plays a role in osmoregulation, and TMAO is an osmolyte generated by gut microbiota from betaine, choline, and carnitine. Betaine and TMAO are biomarkers of cardiovascular outcomes. Two dietary-related compounds, caffeine and trigonelline, are found in coffee beans and are biomarkers of coffee consumption.
Figure 3. Biological roles of metabolites correlated between SBF and plasma. Results from Spearman correlations between SBF and plasma (for metabolite features present in ≥4 matched sample pairs) were input into Mummichog, a Python program for network analysis and metabolite prediction in untargeted metabolomics data sets. Significant features (p < 0.05) were matched to compounds based on common adducts and isotopes; network modules (i.e., subcommunities of biologically interconnected metabolites) were identified based on their “activity scores” (calculated from the number of significant features in the module, as well as the Newman–Girvan modularity Q), and pathway enrichment was estimated using a permutation procedure. (A) Metabolic pathways over-represented among metabolites correlated between SBF and plasma (p < 0.01). (B) Radial plot of biological roles of metabolites identified in network modules, assessed using KEGG BRITE. The inner and outer rings display BRITE functional hierarchies, with the area proportionate to the number of metabolites that fall under each category. Gray boxes outlined in the outer ring show the number of metabolites that belong to each category.

Table 2. Metabolites Strongly Correlated between SBF and Plasma

<table>
<thead>
<tr>
<th>category</th>
<th>putative compound</th>
<th>confidence score</th>
<th>rho</th>
</tr>
</thead>
<tbody>
<tr>
<td>betaine/methionine</td>
<td>homocysteine</td>
<td>3</td>
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<td>betaine</td>
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<td>methionine</td>
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<td>trimethylamine N-oxide</td>
<td>trimethylamine N-oxide</td>
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<td>glutamine</td>
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<td>0.92</td>
</tr>
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<tr>
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<tr>
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<td>0.76</td>
</tr>
<tr>
<td>ATP-associated</td>
<td>creatinine</td>
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</tr>
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<td>phosphocreatine</td>
<td>phosphocreatine</td>
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<tr>
<td>carnitines</td>
<td>acetylcarnitine</td>
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<td>0.84</td>
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<td>trans-2-dodecanoylcarnitine</td>
<td>4</td>
<td>0.83</td>
</tr>
<tr>
<td>decanoylcarnitine</td>
<td>decanoylcarnitine</td>
<td>4</td>
<td>0.81</td>
</tr>
<tr>
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<td>3,5-tetradecadiencarnitine</td>
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<td></td>
<td>LysoPE(18:2)</td>
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<td>coffee-associated</td>
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<td>trigonelline</td>
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<td>polypropylene glycol</td>
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</tr>
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<td>2-(1-propenyl)-delta 1-piperideine</td>
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<td>0.80</td>
</tr>
<tr>
<td>other exogenous</td>
<td>octadecanamide</td>
<td>4</td>
<td>0.88</td>
</tr>
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</table>

*Compound identification confidence score: 4, feature was successfully grouped into a parent ion cluster with a unique database match; 3, feature was successfully grouped, but compound was selected from multiple database matches; 2, feature was not successfully grouped but had a unique database match for [M + H]+, [M + Na]+, or [M + K]+; 1, feature was not successfully grouped, and compound was selected from multiple database matches for [M + H]+, [M + Na]+, or [M + K]+. aAssessed with Spearman correlation.

characterization of detected m/z features without database or library matches. The structural elucidation of these features is challenging due to the lack of reference standards, limited SBF sample volume, and low feature abundances. In addition, a limited number of the annotated metabolites were identified by comparison to authentic reference standards. The remaining metabolites were characterized using an annotation scheme that reduces false identifications through a combination of correlation and adduct/isotope clustering; while this approach has shown to enhance annotation accuracy, additional laboratory analyses, such as ion dissociation and comparison to authentic reference standards, are required for absolute confirmation of identity. We also cannot disentangle the impacts of matrix effects and dilution effects under the current study design.

It is possible that the different sampling sites, i.e., blood draw from the forearm and suction blisters on the thigh, introduced variability in the composition of these fluids, although we do not expect this variability to be significant since plasma is part of systemic circulation. If metabolite concentrations across sampling sites are variable, the interpretation of the metabolite fold changes between SBF and plasma may have been affected. The method for suction blister generation requires a 45 min vacuum application at elevated temperatures; due to this procedure, host responses to blister generation, inflammatory reactions, and wound responses may be observed, resulting in artifacts in the SBF that would not be present in ISF from unperturbed skin. Thus, we cannot dismiss the possibility that some of our findings in SBF (e.g., metabolites elevated in SBF) might be influenced by the injury induced through the suction blister sampling method. Consistent with this hypothesis, a recent study found that plasma and ISF collected via microneedle patches had similar proteomic compositions. In addition, different fluid collection protocols may introduce variability in the metabolites detected. To address this issue in part, future studies will explore the ISF and plasma metabolomes with varying sample dilutions and sampling strategies. Finally, the study had a small sample size, and we were unable to infer how sex, age, and other participant characteristics influenced our results. Nonetheless, the current study supports the use of SBF as a useful fluid for biomarker
monitoring using HRM approaches and provides a framework for future clinical and exposome studies.

## CONCLUSIONS
To our knowledge, this is the first study to compare the human SBF and plasma metabolomes with untargeted HRM profiling. We found that SBF has a distinct metabolite composition that may provide value as a source of biomarkers for diagnostics and monitoring. Our findings suggest that SBF may be a unique source of several biomarkers, including several nucleotides, epidermally derived metabolites, and dietary compounds. Additionally, many clinical biomarkers were well correlated between SBF and plasma, suggesting that SBF has the potential to serve as a surrogate source of biomarkers conventionally detected in plasma. Overall, metabolomic profiling performed in this study provides early evidence that SBF and other alternative biofluids have the potential to serve as a substitute and/or complement to plasma-based biomarker detection in future clinical practice and research studies.

## METHODS

### Obtaining Plasma and SBF Samples
The study was conducted using 10 healthy human volunteers and was approved by the Institutional Review Board (IRB) at the Georgia Institute of Technology. Written informed consent was obtained from all volunteers. Blood samples were taken from the forearm by venipuncture. Skin suction blister fluid was collected from suction blisters generated on the thigh by applying suction at 50−70 kPa below atmospheric pressure at a temperature of 40 °C for ∼45 min until blister formation was complete. See SI for details.

### High-Resolution Metabolomics
SBF samples were diluted from 15 to 50 µL with water. A volume of 50 µL of biofluid (plasma or diluted SBF) was added to 100 µL of acetonitrile, vortexed, and allowed to equilibrate. Proteins were precipitated by centrifugation. Aliquots were analyzed using reverse-phase C18 liquid chromatography (Ultimate 3000, Dionex, Sunnyvale, CA) and Fourier transform mass spectrometry (Q-Exactive, Thermo Scientific, Waltham, MA). Data was extracted using apLCMS and MetaboAnalyst 3.0. Metabolites were identified with analytical standards and accurate mass matching in KEGG and HMDB using custom dataset-wide and feature-specific deconvolution and identification algorithms. See SI for details.

### Statistical Analysis and Metabolite Feature Identification
Statistical analysis, network/pathway analysis, and metabolite set enrichment analysis were performed in RStudio v0.99.48666 and MetaboAnalyst 3.0. Metabolites were identified with analytical standards and accurate mass matching in KEGG and HMDB using custom dataset-wide and feature-specific deconvolution and identification algorithms. See SI for details.

## ASSOCIATED CONTENT

### Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.7b04073.

Methods for fluid collection and sample preparation for analysis; statistical methods used to analyze the untargeted metabolomics data, including fold change and correlation analyses; methods for annotating m/z features based on accurate mass; network and pathway analyses; metabolite set enrichment analysis; Tables S1 and S2; Figure S1 (PDF)

Results from data set-wide annotations listing feature m/z and retention times, clustering assignments by parent ion, adduct types, compound matches in the KEGG database by parent monoisotopic mass, and ion intensities in plasma and SBF study sample (XLSX)

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### Author Contributions
M.M.N. and P.S. contributed equally to this work. M.R.P., G.W.M., P.S., and M.M.N. developed the concept and design of the study. M.R.P., G.W.M., and D.P.J. supervised all research. P.S. collected biological samples from study participants. V.T. and D.I.W. conducted metabolomics assays. M.M.N. performed metabolomics data analysis. M.M.N., P.S., and D.I.W. interpreted results of data analysis. M.M.N. and P.S. wrote the manuscript. M.M.N., P.S., M.R.P., and G.W.M. revised the manuscript. All authors edited and approved the final manuscript.

### Notes
The authors declare no competing financial interest.

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