CRITICAL ILLNESS METABOLOMICS REGARDING INFLAMMATION AND KIDNEY DYSFUNCTION: POST-HOC ANALYSES OF THE VITDAL-ICU TRIAL

CRITICAL ILLNESS METABOLOMICS REGARDING INFLAMMATION AND KIDNEY DYSFUNCTION: POST-HOC ANALYSES OF THE VITDAL-ICU TRIAL

by

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I have reviewed this thesis. It represents work done by the author under my guidance/supervision.

Primary Mentor: Dr. Kenneth B. Christopher

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OVERVIEW

During the Master of Medical Sciences program at Harvard Medical School, I have focused on the metabolomics of critical illness. Critical illness is a life-threatening condition and presents high hospital mortality as much as 20% to 40% (1). One in five Americans dies due to critical illness. Despite specialized care in the intensive care unit (ICU), there are few specific treatments to treat critically ill patients effectively. The reason for this is supposed that the heterogeneity of patients with critical illness (2,3).

Metabolomics research is the large-scale, exhaustive analysis of metabolites that may reflect many reactions influenced by environments and diseases in human body. Metabolomic research is supposed to be able to subdivide heterogeneous patient groups into patient's subtypes according to metabolomic profiles.

Manuscript 1: Acute inflammation is an early nonspecific response to cell stress and injury that is common in almost of critical illness. Recent metabolomic research has not addressed the metabolic response to acute inflammation in general critically ill patients (4). We hypothesized that there is a specific plasma metabolomic profile that represents a response to elevated procalcitonin in critical illness

Manuscript 2: Acute renal failure is a major organ failure in critically ill patients. Previous metabolomic investigations among specific patient groups revealed some metabolites were associated with acute kidney injury (5,6). However, a metabolomic profile related to acute kidney dysfunction in general critically ill patients remains unclear. We hypothesized a specific plasma metabolomic profile is different according to the acute changes of serum creatinine levels in nonspecific patients with critical illness.

Manuscript 1

Procalcitonin Metabolomics in the Critically Ill: a post-hoc metabolomics cohort study of the VITdAL-ICU trial

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ABSTRACT

Background: Procalcitonin is a general biomarker of systemic inflammation and may have importance in the pathophysiology of the systemic inflammation response in critical illness. The metabolomic response to elevated procalcitonin in critical illness is unclear.

Research question: In adults, does the metabolomic response to critical illness differ with elevation of procalcitonin levels?

Study design and methods: We performed a post-hoc metabolomics study of the Correction of Vitamin D Deficiency in Critically Ill Patients (VITdAL-ICU) trial. We analyzed the abundance of 659 metabolites in 1212 plasma samples from 444 subjects at randomization (day 0), day 3 and 7. Serum procalcitonin levels were contemporaneously measured. The relationships between metabolites and procalcitonin levels were assessed via Student's t test, orthogonal partial least square-discriminant analysis, Gaussian graphical models, mediation and linear mixed-effects models with Bonferroni multiple testing correction.

Results: The mean (SD) procalcitonin levels at day 0, day 3, and 7 were 7.32 (29.62) ug/L, 2.93 (9.68) ug/L, and 1.57 (6.62) ug/L, respectively. Multiple metabolites of the dicarboxylate fatty acid, branched chain amino acid, phosphatidylethanolamine, and polyamine metabolite classes were significantly increased with elevated procalcitonin. Further, multiple representatives of long-chain acylcarnitine, acylcholine, lysophospholipid, lysoplasmalogen, and sphingomyelin metabolite classes had significant negative associations with elevated procalcitonin.

Interpretation: Dynamic metabolomic profiles early in critical illness are substantially different in patients with elevations in procalcitonin levels. Such metabolic profile alterations with elevated procalcitonin suggest severe mitochondrial stress, endothelial dysfunction, and immune response dysregulation. The abundance of acylcarnitine, dicarboxylic fatty acid, and branched chain amino acid metabolites specifically indicate activation of branched chain amino acid dehydrogenase and a metabolic shift with increased procalcitonin.

Keywords

Metabolomics, Procalcitonin, Critical illness, Acylcarnitine, Dicarboxylate fatty acids

List of non-standard abbreviations

VITdAL-ICU trial - Correction of Vitamin D Deficiency in Critically Ill Patients trial; OPLS-DA - orthogonal partial least square-discriminant analysis; CV-ANOVA - cross-validation analysis of variance

INTRODUCTION

Acute inflammation is an early nonspecific response to cell stress and injury that underlies much of critical illness physiology. The 116-amino acid polypeptide Procalcitonin (PCT) is a general biomarker of the severity of systemic inflammation. Elevated PCT is noted in bacterial, fungal and parasitic infection, sepsis, trauma, surgery, cardiogenic shock, autoimmune disease and severe COVID-19 (1,2). PCT is a soluble protein with a 25-30 hour half-life that is detectable in the blood of healthy adults (<0.05 ug/L) (3). PCT rapidly increases 1000 fold with severe critical illness (4). The release of PCT into circulation is stimulated by microbial toxins as well as the immune response (IL-6, TNFα, IL-1β) and attenuated by IFN-γ (5). The major source of circulating PCT during inflammation is the parenchymal tissue, especially liver, lung, kidney, adipose and muscle (6). Clinically, PCT measurement is used to diagnose and stratify risk of bacterial sepsis and monitor response to antibiotics for reduction of antibiotics prescription (7–9). Additionally, PCT is associated with the severity of illness and predicts clinical outcomes (10).

Beyond its status as a biomarker, experiments showing PCT is important in the systemic inflammation response have led to scientists to propose that PCT may have a detrimental role in the host response to inflammation in critical illness. PCT is shown to enhance the inflammatory response and stimulate the surface expression of CD16 on human neutrophils and CD14 on lymphocytes (7,11). In experimental studies, PCT exposure leads to endothelial barrier function impairment and hepatocyte dysfunction (12,13). Further, experimental models of sepsis show the presence of PCT worsens illness severity and outcomes (14).

Metabolomic studies performed on blood collected early in critical illness show a profoundly disturbance of metabolic homeostasis that reflects illness severity and is predictive of adverse outcomes (15). But such work has not addressed the metabolic response to inflammation in critical illness (16). Therefore, we studied differences in PCT levels with regard to changes in metabolism during critical illness. We used global metabolomic profiling to capture a diverse range of metabolites that are measured in plasma, reflecting multiple metabolism pathways. We hypothesize that there is a specific metabolomic profile that represents a response to elevated PCT in critical illness. We performed global metabolomic profiling that resulted in 659 metabolites from 1212 plasma samples that represent three time points in 444 subjects collected during the VITdAL-ICU trial (17,18). We assessed the effect of increased PCT on changes in individual metabolites and metabolic pathways over three time points early in the course of critical illness. Further, we identified a group of specific metabolites that change in unison with increased PCT.

METHODS

Detailed trial and metabolomics methods are presented in Supplemental Methods. Briefly, the VITdAL-ICU trial (NCT01130181) randomized 475 critically ill adult subjects to vitamin D3 or placebo once at a dose of 540,000 IU followed by 90,000 IU monthly (17). The primary study outcome was hospital length of stay. Whole blood was collected at randomization (day 0), day 3 and day 7. Written informed consent was obtained at the time of VITdAL-ICU trial enrollment. The post-hoc metabolomics study protocol was granted approval by the Partners Human Research Committee at the Brigham and Women's Hospital (Protocol # 2015P002766).

Our exposure of interest was serum PCT measured at day 0. The PCT level of <0.5 ug/L was assigned as the referent that is considered as a cut-off value to discontinue antibiotics in critical illness (8, 9). Frozen plasma was available for analysis in 453 trial subjects. We excluded 9 subjects who did not have serum PCT measured at day 0. To generate metabolomics data, a total of 1212 plasma samples from 444 subjects at day 0, 401 subjects at day 3 and 367 subjects at day 7 were analyzed using four ultra high-performance liquid chromatography/ tandem accurate mass spectrometry methods by Metabolon, Inc. in 2017 (**Supplemental Figure 1**) (18). Metabolomic profiling identified 983 plasma metabolites. We reduced baseline noise by removing metabolites with the lowest interquartile range of variability, leaving 659 metabolites. Individual metabolite raw area count data was normalized, underwent cube root transformation and then Pareto scaling to generate abundance data that were on the same scale and followed an approximate normal distribution.

For univariate analysis of day 0 data, Student's t test was used to identify metabolites that are associated with a dichotomized PCT measure (PCT <0.5 ug/L versus PCT ≥0.5 ug/L) applying a Bonferroni multiple testing correction threshold of P-value $<7.59 \times 10^{-5}$ (0.05/659 metabolites per plasma sample) using MetaboAnalyst (19). Day 0 data were also analyzed using orthogonal partial least square-discriminant analysis (OPLS-DA), a supervised method to assess the significance of classification discrimination (SIMCA 15.0 Umetrics, Umea, Sweden). We performed permutation testing to validate the OPLS-DA model. We employed sevenfold cross-validation analysis of variance (CV-ANOVA) to determine OPLS-DA model significance. A Receiver Operating Characteristic (ROC) curve was calculated from class-belonging values predicted by the OPLS-DA model. We produced a misclassification table of the proportion of correctly classified observations (PCT <0.5 ug/L vs PCT ≥0.5 ug/L) in the day 0 data.

For repeated measures data, the association between relative abundance of individual metabolites (outcome) and PCT levels (as a continuous exposure) at day 0, 3 and 7 were determined utilizing linear mixed-effects models correcting for age, sex, baseline 25(OH)D, absolute increase in 25(OH)D at day 3, SAPS II, admission diagnosis, plasma day (as controlling the effects of time) and individual subject (as the random-intercept). We excluded 25 trial subjects who did not have serum 25(OH)D measured at day 3 as this data is essential to adjust for intervention response. A total of 1187 plasma samples from 419 subjects at day 0, 401 subjects at day 3 and 367 subjects at day 7 were analyzed with linear mixed-effects models. To identify all significant mixed-effects associations we utilized multiple testing correction based on

the Benjamini-Hochberg procedure to adjust the false-discovery rate (FDR) to 0.05 (20). All mixed-effects models were analyzed using STATA 14.1MP (College Station, TX). We employed rain plots to clearly visualize graded effect size, significance, clustering and trends across several PCT cut-off levels (21). Rain plots were produced based on hierarchical clustering in R-3.6.2.

To identify PCT-specific modules from metabolite abundance data, we applied Gaussian graphical models (GGMs) using the metabolomic data from day 0 using the GeneNet R package, version 1.2.13 in R-3.6.2 (22). Modules are identified by reconstruction of pathway reactions derived from metabolomics data. GGMs are determined utilizing partial pairwise Pearson correlation coefficients following the removal of the effects of all other metabolites and covariates (23). We inferred a PCT-specific network (PCT <0.05 vs ≥0.05 ug/L) for relative metabolite abundance. We included age, sex, SAPS II, admission diagnosis, and baseline 25(OH)D as covariates into the model. We allocated edges between metabolites if both their Pearson correlations and partial correlations remained statistically significant at P-value <0.05 subsequent to Bonferroni correction for 659 metabolites (22).

As the liver is a lymphoid organ, age is an important regulator of inflammation, and PCT levels are associated with obesity, we evaluated a potential mediating effect of bilirubin, age or body mass index on the association between PCT and individual metabolite abundance adjusted for age, sex, baseline 25(OH)D, absolute increase in 25(OH)D, SAPS II and admission diagnosis. Analyses were performed on each of the 659 metabolites at day 0 using the R package mediation (24) to obtain bootstrap P values (N = 2000 samples) for the mediation effect of age or for bilirubin. Significant

mediation was present if the P-value was <0.01 and if ≥10% of the association was mediated through bilirubin levels, age or body mass index.

RESULTS

In the analytic cohort (N=444), we found the mean (SD) of PCT at day 0 was 7.32 (29.62) ug/L, at day 3 was 2.93 (9.68) ug/L and at day 7 was 1.57 (6.62) ug/L. Baseline characteristics of the cohort were balanced between subjects grouped by PCT level for age, SAPS II score, 25(OH)D level at day 0, intervention status and the absolute change in 25(OH)D level at day 3. Differences existed with respect to sex, C-reactive protein, Day 0 total bilirubin and creatinine, ICU type, and admission diagnosis category (**Table 1, Supplemental Table 1**). The overall 28-day mortality of the 444 subject analytic cohort was 25.7%.

In day 0 plasma samples (N=444), significant crude differences exist in 301 individual metabolites (all Bonferroni corrected P-value <7.59 × 10-5) in subjects with or without PCT ≥0.5 ug/L dominated by increases of multiple metabolite members of the branched chain amino acid, short-, medium-chain acylcarnitine, dicarboxylate fatty acid pathways and decreases in metabolites from the long-chain acylcarnitine, lysophosphatidylcholine and sphingomyelin pathways. Regarding differences in metabolomic profiles of subjects with or without PCT ≥0.5 ug/L at day 0, the OPLS-DA model had acceptable predictability (Q2 value 0.422). Confirmation of the stability and robustness of the OPLS-DA model was shown by the permutation test (Q2 intercept of -0.187, P-value ≤0.05) with a negative permutation Q2 intercept indicating model validity (**Supplemental Table 2**). The cross-validation procedure showed that the groups with or without PCT ≥0.5 ug/L were significantly separated (CV-ANOVA P-value <0.001). The ROC analysis showed the predictive ability of the OPLS-DA model was excellent (AUC = 0.91). Further, the model showed good classification performance with 82.9% of

cases with PCT ≥0.5 ug/L were correctly classified (sensitivity of 85.3%, specificity of 78.1%).

In the repeated measure data, mixed-effects modeling of 1187 plasma samples collected at day 0, 3 and 7 from 419 VITdAL-ICU trial subjects, 155 metabolites had significantly positive associations with PCT. The metabolites were dominated by increases in multiple representatives of each of the following pathways: branched chain amino acids, dicarboxylate fatty acids, phosphatidylethanolamines, and polyamines (**Summarized in Table 2, full data in Supplemental Table 3, Figures 1 & 2**). Eighty-one metabolites had a significant negative association with PCT, including multiple representatives of acyl choline, long-chain acylcarnitine, lysophospholipid such as lysophosphatidylcholines, lysoplasmalogen, and sphingomyelin pathway metabolites (**Summarized in Table 3, full data in Supplemental Table 4, Figure 1**).

We next performed individual mixed-effects models on the 1187 day 0, 3 and 7 plasma samples in subjects grouped by PCT cut points (0.05 - <0.10 ug/L, 0.10 - <0.25 ug/L, 0.25 - <0.50 ug/L, 0.50 - <1.00 ug/L, 1.00 - <2.00 ug/L, 2.00 - <10.00 ug/L or ≥10.00 ug/L) relative to referent subjects with PCT <0.05 ug/L, a level found in healthy subjects (**Supplemental Table 1**) (24). The rain plots (**Figure 1 & 2**) showed the separation of highlighted acylcarnitine and BCAA metabolites that are increased (red) or decreased (blue) in subjects with increasing PCT relative to subjects with PCT <0.05 ug/L. Greater significance is shown by increased size of the circles. With increasing PCT we find a monotonic increase in the effect size and significance of dicarboxylate fatty acids and phosphatidylethanolamines, while a monotonic decrease in the effect size and significance is present in acylcholines, lysophospholipids (**Supplemental**

Figure 2-5). Long-chain acylcarnitines showed significantly decreased effect sizes with increasing PCT whereas short-chain acylcarnitines had monotonic increases in effect size and significance (**Figure 1**).

To explore mechanistic insight into our observation of increased BCAA metabolites with increasing PCT, we present unadjusted metabolite abundance data at day 0, 3 and 7 for subjects with PCT<0.5 ug/L or ≥0.50 ug/L (**Supplemental Figure 6**). Boxplots show relative abundance of plasma BCAAs, branched-chain keto acids, metabolites downstream from branched chain amino acid dehydrogenase (BCKDH) and BCAA-derived carnitines. The abundance of BCAA metabolites downstream from the BCKDH enzyme complex increase at days 3 and 7 in subjects with PCT ≥0.50 ug/L but not with PCT<0.5 ug/L (**Supplemental Figure 6 Panels J-T**).

We next explored PCT-specific relationships between metabolites. With Gaussian graphical models (GGMs) we measured pairwise correlations in metabolites that have similar effects. The GGM analyses revealed six PCT-specific functional modules at day 0 (**Supplementary Table 5**). Similar to the mixed effects analyses, metabolism of androgenic steroids, polyamines, BCAAs and dicarboxylate fatty acids are prominently featured in the PCT-specific GGM modules. Metabolites within in each functional module were increased with increased PCT nearly in unison as well as having biological and functional similarity (i.e. in **Supplementary Table 5** Module C, 5 of 6 members are polyamide metabolites).

We finally focused on the potential mediation of the relationship between metabolite abundance and PCT levels by liver function, body mass index or age. Mediation analyses in day 0 data revealed no influence of body mass index or age on

associations between PCT levels and all 659 metabolites. With regard to bilirubin, mediation analyses in day 0 data revealed a significant influence on associations between PCT levels and bilirubin of 120 of the 659 individual metabolites (all P-values were <0.01 and proportion mediated over 10% using 2000 bootstrap samples). Ninety-four of these mediated metabolites were also identified in our mixed-effects analysis as significantly changed with PCT levels (**Supplemental Figure 7**).

DISCUSSION

The underlying mechanisms of alterations of metabolomic disruption during inflammation remain an elegant enigma. Using multiple analytic approaches, our novel metabolomics study detected groups of metabolites along similar sub-pathways with strong associations with PCT levels. In the setting of an elevated PCT, our data highlights increases in dicarboxylate fatty acids, phosphatidylethanolamines, and polyamines metabolite classes and decreases in long-chain acylcarnitines, acylcholines, lysophosphatidylcholines, lysoplasmalogens, and sphingomyelins. Further, we illustrate how groups of metabolites with similar PCT associations form modules which may have relevance to the biological interpretation of our metabolomics observations. Our data suggest that knowledge of such patterns and their biological effects are critical for understanding the role of inflammation in general and PCT specifically.

To put our findings into context, the known properties of highlighted metabolites are discussed as a guide for data interpretation. With increased PCT we find metabolic evidence of mitochondrial dysfunction. We find a specific pattern of change in acylcarnitines with increased PCT where short-chain acylcarnitines are elevated and long-chain acylcarnitines are decreased (**Figure 1**). Primarily released from the liver, plasma short-chain acylcarnitines (C2-C7) are due to incomplete mitochondrial fatty acid β-oxidation and indicative of impaired mitochondrial function (26). On the other hand, medium- (C8-C12) and long-chain acylcarnitines (C14-) are produced through mitochondrial β-oxidation (27). The specific pattern of change in acylcarnitines may indicate incomplete fatty acid β-oxidation that only shortened the long-chain fatty acids. We also observe an increase in dicarboxylic fatty acids known to be produced by fatty

acid omega oxidation (ω-oxidation) when incomplete fatty acid β-oxidation occurs in the setting of mitochondrial dysfunction (28). The cytochrome P450 (CYP4F) that catalyzes the first step of fatty acid omega oxidation is induced by the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α (29). Such circulating dicarboxylic fatty acids are shown to increase in response to starvation and critical illness (30). Our observation of decreases in long-chain acylcarnitines and increases in both plasma short-chain acylcarnitines and dicarboxylic fatty acids with increases in PCT may reflect less efficient fatty acid β-oxidation through impaired mitochondrial bioenergetics associated with increased inflammation (31). The mediation of the association between PCT level and metabolite abundance by serum total bilirubin underscores the importance of the liver in metabolism, immunity, inflammation and PCT induction (32).

Catabolic stress liberates amino acids into the circulation by endogenous protein breakdown including the branched-chain amino acids (BCAA), leucine, isoleucine and valine (33). During inflammation, BCAAs are preferentially transported to the liver over the muscle (34). BCAAs are metabolized to acetyl-CoA or succinyl-CoA when mitochondrial fatty acid β-oxidation is incomplete. The irreversible and rate-limiting step of BCAA catabolism is the branched-chain α-ketoacid dehydrogenase (BCKDH) complex in the mitochondrial matrix (35) (**Supplemental Figure 6**). Experimental animal evidence shows that BCKDH is rapidly activated by acute nutrient deprivation, circulating BCAA excess, exercise, endotoxin, IL-1β and TNFα (36–40). Limited evidence suggests that circulating BCAA excess and mitochondrial BCAA catabolism are measurable in healthy humans under exercise stress and also in the critically ill (41–43).

We observe circulating BCAA catabolic metabolites distal to BCKDH are significantly increased with increasing PCT, suggesting BCKDH activation (**Table 2, Figure 2, Supplemental Figure 6**). Further, we find that short-chain acylcarnitines is increased with increases in PCT (**Table 2, Figure 1, Supplemental Figure 6**). The observed increases of circulating short-chain acylcarnitines, dicarboxylate fatty acids and BCAA catabolic metabolites are all indicative of a metabolic shift. Experimental evidence in healthy humans supports that such a metabolic shift is an adaptive response to endotoxin via alteration of mitochondrial bioenergetics (44).

We find that higher PCT is associated with increased levels of circulating metabolites of polyamine catabolism (**Supplementary Table 5, highlighted in Module C**). Cellular polyamines (spermidine and spermine) are tightly regulated polycations that regulate cell growth and proliferation (45). Cellular polyamine synthesis is up-regulated during bacterial infections and inflammation (46). During such inflammatory stress, the catabolic enzyme spermidine/spermine N1-acetyltransferase (SSAT) is induced by TNFα. Induced SSAT increases polyamide catabolism and the exit of intracellular polyamine metabolites into the circulation. Such decreases in the concentration of intracellular polyamines lead to slower cell growth rates allowing for potential cell repair or increased apoptosis during inflammation (47).

Further, we demonstrate that increased PCT is associated with increases in phosphatidylethanolamines and decreases in lysophospholipids (**Supplemental Table 3 & 4**). Phosphatidylethanolamines are present on microparticle surfaces of the endothelium and white blood cells are liberated from endothelial cells following exposure to oxidative stress and found in plasma following experimental sepsis (48,49).

Lysophosphatidylcholines, which are members of lysophospholipids, are proinflammatory lipids that activate monocytes, macrophages and T cells. Lower levels of lysophosphatidylcholines are associated with severity of community-acquired pneumonia and sepsis (50). The increased phosphatidylethanolamines and decreased lysophospholipids with increased PCT observed in our study may reflect endothelial dysfunction from inflammation or direct PCT exposure and indicate dysregulation of the immune response in the setting of more intense inflammation, respectively (12).

Our methodology has multiple strengths. Linear mixed-effects models are vigorous analysis tools for metabolomics studies with repeated time points and multiple clinical variables (51). Our approach allows for a focus on metabolites that change relative to PCT rather than simply change with the course of critical illness or trial intervention (52). To limit false positive observations, we conservatively adjusted our mixed-effects significance threshold to account for 659 multiple comparisons. The use of the GGM identification algorithm enhances our association analyses (22). Further, prior studies show the importance of PCT to the response to severe critical illness thus increasing the relevance and biological plausibility of our observations (5,7,11,14).

Our study does have potential limitations. In longitudinal data, patients who were discharged early from the ICU or died rapidly provided fewer samples than patients who survived in the ICU over seven days. We applied linear mixed-effects models that could analyze whole longitudinal samples, including data from a single time point, to demonstrate significant linear associations between PCT and metabolites. Nevertheless, it may result in the inaccuracy of the effects of time. Despite multivariable adjustment, our use of nonrandomized comparisons is subject to bias as subjects with

increases in PCT may systematically differ and be potential risk for residual confounding. Especially, the ICU types where patients were admitted to were markedly different between PCT levels of patients. However, ICU settings depended on the physician's decision and bed controlling in the hospital. Thus, we adjusted the admission diagnosis in mixed-effects models to control pathophysiologic backgrounds of patients rather than ICU types. Moreover, our analytic cohort included subjects who had chronic kidney disease and renal failure and required hemodialysis. Renal function is closely associated with the emission of metabolites and metabolism. Although we adjusted SAPS II, which includes factors of renal function, our findings might be affected by renal function in patients. Additionally, we used actual response of serum 25(OH)D values at day 3 to intervention rather than the randomized treatment arms. Vitamin D responsiveness was significantly correlated with the alterations of vitamin D receptor genes and mRNA expression (53). In the VITdAL-ICU trial, only half of the participants assigned to the high dose oral vitamin D3 group showed the expected increase in serum 25(OH)D levels ≥ 30 ng/mL. The fewer proportion of participants with responsiveness to vitamin D biases the intention-to-treat analyses to the null (54). Besides, we performed a post-hoc analysis of plasma samples with correction for multiple testing. Thus, our finding should be considered hypothesis generating. Our study population is heterogenous and increased PCT may be present for different reasons. Further, our study of White critically ill subjects from a single large academic medical center may have limited generalizability. Finally, while the highlighted metabolites have known functional and biological relevance, the clinical significance of a change in metabolite abundance may be unclear.

INTERPRETATION

Taken together, our data indicate that inflammation is associated with alteration of energy utilization by specific metabolic pathways in critical illness. Early critical illness represents a state of nutrient deprivation, oxidative stress and mitochondrial dysfunction which compromise tissue metabolic needs. Circulating metabolites provide an assessment of internal energy states and energy substrate selection. Our findings provide convergent evidence that PCT related inflammation alters mitochondrial bioenergetics. Identifying circulating metabolic information over time is a first step towards understanding the dynamics of energy utilization in critical illness and the metabolomic effects of inflammation.

TAKE-HOME POINT

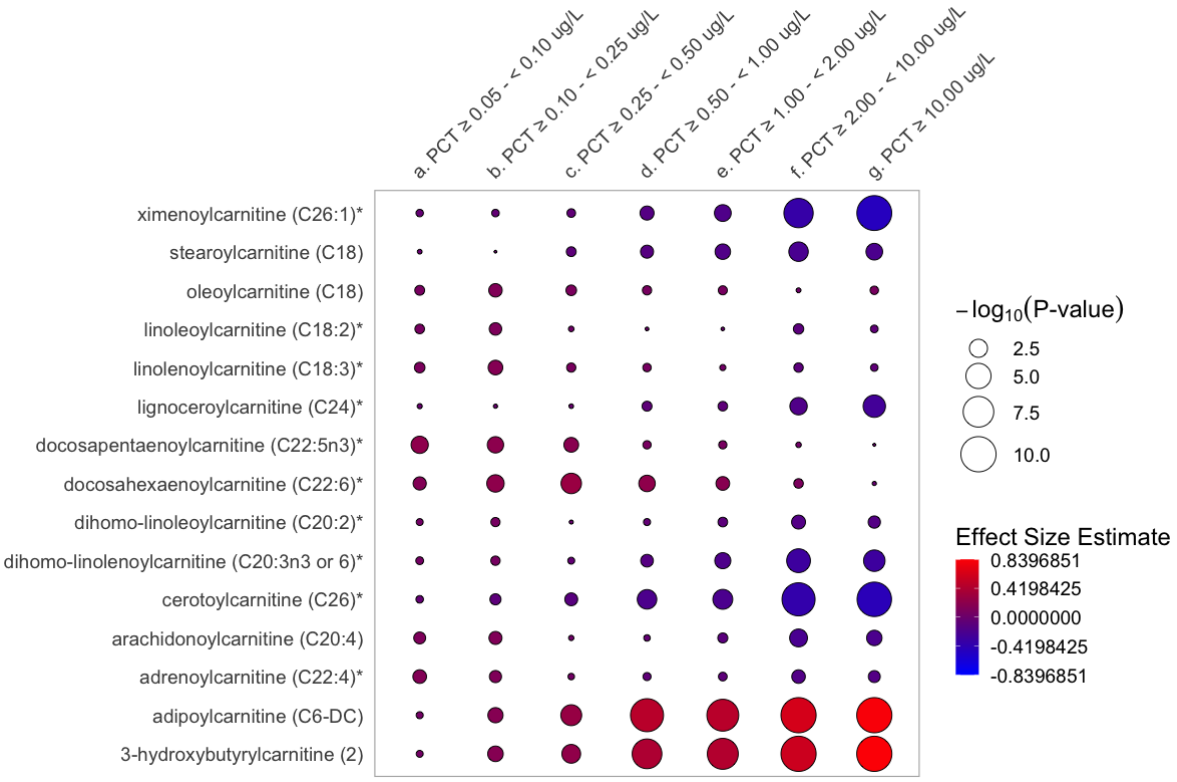
Study Question: Do critically ill adults have different critical illness metabolism profiles with increased procalcitonin?

Results: Metabolite profiles in the critically ill with elevated procalcitonin drastically differ along coordinated pathways, most notably increases in dicarboxylic fatty acid and branched chain amino acid metabolites and decreases in long-chain acylcarnitine.

Interpretation: Our data suggests that with elevated procalcitonin, energy utilization is dramatically altered indicating a metabolic shift with specific activation of mitochondrial branched chain amino acid dehydrogenase.

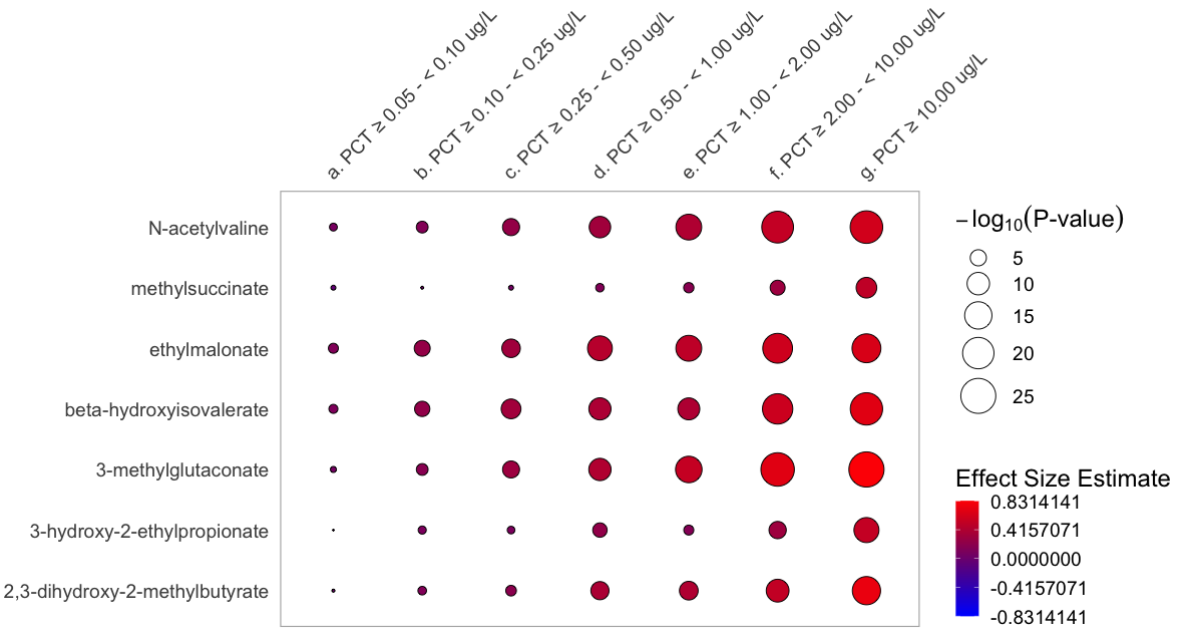
FIGURES

Figure 1. Rain Plot of repeated measures acylcarnitine metabolomics data (day 0, 3 and 7) relative to PCT level



Correlations between individual acylcarnitine metabolites and PCT level groups at day 0, 3 or 7 were determined utilizing linear regression models correcting for age, SAPS II, admission diagnosis, 25(OH)D at day 0 and for absolute change in 25(OH)D level at day 3. The magnitude of beta coefficient estimates is shown by a color fill scale and the corresponding significance level ($-\log_{10}$(P-value)) is represented by size of the circle. The intensity of the red fill color represents an increase in effect size for that metabolite in PCT level groups compared to subjects with PCT <0.05 ug/L. The intensity of the blue fill color represents a decrease in effect size for that metabolite in PCT level groups compared to subjects with PCT <0.05 ug/L. Statistical significance is the multiple test-corrected threshold of $-\log_{10}$(P-value) > 1.45 which is equivalent to FDR < 0.05.

Figure 2. Rain Plot of repeated measures BCAA metabolomics data (day 0, 3 and 7) relative to PCT level



Correlations between individual BCAA metabolites and PCT level groups at day 0, 3 or 7 were determined utilizing linear regression models correcting for age, SAPS II, admission diagnosis, 25(OH)D at day 0 and for absolute change in 25(OH)D level at day 3. The magnitude of beta coefficient estimates is shown by a color fill scale and the corresponding significance level (-log10(P-value)) is represented by size of the circle. The intensity of the red fill color represents an increase in effect size for that metabolite in PCT level groups compared to subjects with PCT <0.05 ug/L. Statistical significance is the multiple test-corrected threshold of -log10(P-value) > 1.45 which is equivalent to FDR < 0.05.

TABLES

Table 1. Analytic Cohort Characteristics by Day 0 Procalcitonin levels

Characteristic	Day 0 PCT		Total	P-value
	<0.50 ug/L	≥ 0.50 ug/L		
No.	187	257	444	
Age years Mean (SD)	63.5 (15.3)	65.8 (14.1)	64.8 (14.7)	0.09
Female No. (%)	78 (42)	77 (30)	155 (35)	0.01
Charlson Comorbidity Index Mean (SD)	2.5 (2.0)	3.4 (2.2)	3.1 (2.2)	<0.001
SAPS II Mean (SD)	32.6 (16.7)	34.2 (14.4)	33.5 (15.4)	0.29
C-reactive protein Day 0(ug/mL) Mean (SD)	86.8 (73.2)	153.1 (89.8)	125.2 (89.4)	<0.001
Day 0 25(OH)D(ng/mL) Mean (SD)	14.6 (6.3)	13.3 (10.1)	13.9 (8.7)	0.13
Vitamin D_3 Intervention No. (%)	83 (44)	136 (53)	219 (49)	0.076
Change in 25(OH)D Day 0 to Day 3(ng/mL) Median [IQR]	2.8 [-0.4, 25.1]	3.3 [0.1, 12.2]	3.1 [0, 16.7]	0.34
Total Bilirubin Day 0(mg/dL) Mean (SD)	0.8 (0.9)	2.1 (3.3)	1.6 (2.6)	<0.001
Creatinine Day 0(mg/dL) Mean (SD)	1.0 (0.7)	1.7 (1.1)	1.4 (1.0)	<0.001
ICU				<0.001
Anesthesia ICU No. (%)	29 (16)	54 (21)	83 (19)	
Cardiac Surgery ICU No. (%)	28 (15)	102 (40)	130 (29)	
Medical ICU No. (%)	30 (16)	69 (27)	99 (22)	
Neurological ICU No. (%)	91 (49)	18 (7)	109 (25)	
Surgical ICU No. (%)	9 (5)	14 (5)	23 (5)	
28-day mortality No. (%)	30 (16)	84 (33)	114 (26)	<0.001

Table 2. Metabolites significantly increased with increased Procalcitonin over days 0-7

Metabolite	β Coefficient	P-value	FDR adjusted P-value	-log$_{10}$p	Super Pathway	Sub Pathway
N-acetylvaline	1.53E-03	3.75E-03	1.48E-02	2.43	Amino Acid	BCAA Metabolism
ethylmalonate	1.55E-03	8.44E-03	2.82E-02	2.07	Amino Acid	BCAA Metabolism
beta-hydroxyisovalerate	1.75E-03	2.87E-03	1.20E-02	2.54	Amino Acid	BCAA Metabolism
3-methylglutaconate	1.91E-03	2.08E-03	9.09E-03	2.68	Amino Acid	BCAA Metabolism
3-hydroxy-2-ethylpropionate	2.13E-03	3.24E-04	1.94E-03	3.49	Amino Acid	BCAA Metabolism
2,3-dihydroxy-2-methylbutyrate	3.67E-03	4.16E-07	1.10E-05	6.38	Amino Acid	BCAA Metabolism
methylsuccinate	4.13E-03	1.56E-09	8.58E-08	8.81	Amino Acid	BCAA Metabolism
3-hydroxybutyrylcarnitine (C4-OH)	1.80E-03	1.31E-02	3.88E-02	1.88	Lipid	Acylcarnitine
adipoylcarnitine (C6-DC)	2.17E-03	3.16E-03	1.30E-02	2.50	Lipid	Acylcarnitine
adipate	1.88E-03	1.60E-02	4.55E-02	1.80	Lipid	Fatty Acid, Dicarboxylate
suberate (C8-DC)	2.02E-03	7.00E-03	2.44E-02	2.15	Lipid	Fatty Acid, Dicarboxylate
3-hydroxyadipate*	2.22E-03	8.93E-03	2.90E-02	2.05	Lipid	Fatty Acid, Dicarboxylate
heptenedioate (C7:1-DC)*	2.36E-03	3.78E-03	1.48E-02	2.42	Lipid	Fatty Acid, Dicarboxylate
octadecanedioate (C18)	2.49E-03	4.98E-03	1.86E-02	2.30	Lipid	Fatty Acid, Dicarboxylate
dodecenedioate (C12:1-DC)*	2.56E-03	3.57E-03	1.45E-02	2.45	Lipid	Fatty Acid, Dicarboxylate
octadecadienedioate (C18:2-DC)*	2.62E-03	9.02E-04	4.63E-03	3.04	Lipid	Fatty Acid, Dicarboxylate
octadecenedioate (C18:1-DC)*	2.63E-03	2.17E-03	9.43E-03	2.66	Lipid	Fatty Acid, Dicarboxylate
hexadecanedioate (C16)	3.17E-03	3.33E-04	1.98E-03	3.48	Lipid	Fatty Acid, Dicarboxylate
3-methyladipate	3.19E-03	3.15E-05	3.15E-04	4.50	Lipid	Fatty Acid, Dicarboxylate
dodecanedioate (C12)	3.23E-03	2.52E-04	1.55E-03	3.60	Lipid	Fatty Acid, Dicarboxylate
tetradecanedioate (C14)	3.85E-03	1.07E-05	1.36E-04	4.97	Lipid	Fatty Acid, Dicarboxylate
hexadecenedioate (C16:1-DC)*	4.23E-03	1.23E-06	2.47E-05	5.91	Lipid	Fatty Acid, Dicarboxylate

Note: Using repeated measures data (day 0, 3 and 7), the association between relative quantitation of each individual metabolite noted above and Procalcitonin levels over time were determined utilizing linear mixed-effects models correcting for age, sex, baseline 25(OH)D, absolute increase in 25(OH)D, Simplified Acute Physiology Score (SAPS) II, plasma day, admission diagnosis and individual subjects (as the random-intercept). All significant mixed-effects associations have Benjamini-Hochberg adjusted P-value <0.05. BCAA is Branched-Chain Amino Acids inclusive of Leucine, Isoleucine and Valine. For the Acylcarnitines sub pathway: a capital C is followed by the number of carbons within the fatty acyl group attached to the carnitine. A colon followed by a number is one or more unsaturated carbons in the acylcarnitine ester (i.e. C10:1 is a monounsaturated C10 acylcarnitine). OH following the carbon number is a hydroxylic acylcarnitine. DC following the carbon number is a dicarboxylic acylcarnitine.

Table 3. Metabolites significantly decreased with increased Procalcitonin over days 0-7

Metabolite	β Coefficient	P-value	FDR adjusted P-value	-log$_{10}$p	Super Pathway	Sub Pathway
linoleoylcholine*	-3.37E-03	1.47E-04	9.91E-04	3.83	Lipid	Acyl Choline
dihomo-linolenoyl-choline	-3.43E-03	1.32E-04	9.33E-04	3.88	Lipid	Acyl Choline
docosahexaenoylcholine	-3.45E-03	5.45E-05	4.92E-04	4.26	Lipid	Acyl Choline
palmitoylcholine	-3.46E-03	7.56E-05	6.15E-04	4.12	Lipid	Acyl Choline
oleoylcholine	-3.54E-03	7.36E-05	6.07E-04	4.13	Lipid	Acyl Choline
stearoylcholine*	-3.60E-03	1.26E-04	9.09E-04	3.90	Lipid	Acyl Choline
arachidonoylcholine	-3.91E-03	4.38E-06	6.82E-05	5.36	Lipid	Acyl Choline
oleoylcarnitine (C18)	-1.58E-03	1.68E-02	4.70E-02	1.78	Lipid	Acylcarnitine
stearoylcarnitine (C18)	-1.85E-03	3.27E-03	1.34E-02	2.49	Lipid	Acylcarnitine
adrenoylcarnitine (C22:4)*	-1.95E-03	8.98E-03	2.90E-02	2.05	Lipid	Acylcarnitine
docosapentaenoylcarnitine (C22:5n3)*	-2.05E-03	1.02E-02	3.18E-02	1.99	Lipid	Acylcarnitine
dihomo-linoleoylcarnitine (C20:2)*	-2.11E-03	2.27E-03	9.77E-03	2.64	Lipid	Acylcarnitine
linoleoylcarnitine (C18:2)*	-2.20E-03	8.96E-04	4.63E-03	3.05	Lipid	Acylcarnitine
linolenoylcarnitine (C18:3)*	-2.32E-03	1.57E-03	7.22E-03	2.80	Lipid	Acylcarnitine
docosahexaenoylcarnitine (C22:6)*	-2.49E-03	1.44E-03	6.84E-03	2.84	Lipid	Acylcarnitine
lignoceroylcarnitine (C24)*	-2.59E-03	2.58E-06	4.36E-05	5.59	Lipid	Acylcarnitine
dihomo-linolenoylcarnitine (C20:3n3 or 6)*	-2.60E-03	1.76E-04	1.15E-03	3.75	Lipid	Acylcarnitine
arachidonoylcarnitine (C20:4)	-2.71E-03	1.34E-04	9.43E-04	3.87	Lipid	Acylcarnitine
cerotoylcarnitine (C26)*	-2.88E-03	5.01E-07	1.27E-05	6.30	Lipid	Acylcarnitine
ximenoylcarnitine (C26:1)*	-3.49E-03	1.03E-08	3.99E-07	7.99	Lipid	Acylcarnitine
1-palmitoyl-GPA (16:0)	-1.97E-03	9.25E-03	2.95E-02	2.03	Lipid	Lysophospholipid
1-palmitoyl-GPI* (16:0)	-1.98E-03	6.76E-03	2.37E-02	2.17	Lipid	Lysophospholipid
1-linolenoyl-GPC (18:3)*	-2.32E-03	1.30E-03	6.32E-03	2.89	Lipid	Lysophospholipid
1-palmitoleoyl-GPC* (16:1)*	-2.60E-03	1.41E-05	1.66E-04	4.85	Lipid	Lysophospholipid
1-arachidonoyl-GPC* (20:4)*	-2.61E-03	1.34E-06	2.60E-05	5.87	Lipid	Lysophospholipid
2-palmitoyl-GPC* (16:0)*	-2.78E-03	9.30E-06	1.20E-04	5.03	Lipid	Lysophospholipid
1-lignoceroyl-GPC (24:0)	-4.03E-03	7.68E-09	3.68E-07	8.11	Lipid	Lysophospholipid

Note: Using repeated measures data (day 0, 3 and 7), the association between relative quantitation of each individual metabolite noted above and Procalcitonin levels over time were determined utilizing linear mixed-effects models correcting for age, sex, baseline 25(OH)D, absolute increase in 25(OH)D, Simplified Acute Physiology Score (SAPS) II, plasma day, admission diagnosis and individual subjects (as the random-intercept). All significant mixed-effects associations have Benjamini-Hochberg adjusted P-value <0.05. BCAA is Branched-Chain Amino Acids inclusive of Leucine, Isoleucine and Valine. For the Acylcarnitines sub pathway: a capital C is followed by the number of carbons within the fatty acyl group attached to the carnitine. A colon followed by a number is one or more unsaturated carbons in the acylcarnitine ester (i.e. C10:1 is a monounsaturated C10 acylcarnitine). GPA is glycerophosphate; GPI is glycerophosphatidylinositol; GPC is glycerophosphorylcholine.

SUPPLEMENTARY MATERIAL

Supplemental Tables

Supplemental Figures

Supplemental Figure 6. Relative abundance of plasma metabolites in branched chain amino acids

Supplemental Figure 7. Proportion mediated by total serum bilirubin for metabolites

Supplemental Table 1. Additional Cohort Characteristics

Characteristic	Procalcitonin at Day 0								Total	P-value
	0.00 - <0.05 ug/L	0.05 - <0.10 ug/L	0.10 - <0.25 ug/L	0.25 - <0.50 ug/L	0.50 - <1.00 ug/L	1.00 - <2.00 ug/L	2.00 - <10.00 ug/L	≥10.00 ug/L		
No.	20	43	74	50	75	50	85	47	444	
Age years Mean (SD)	58.9 (15.3)	61.0 (18.4)	65.2 (14.9)	64.7 (12.6)	66.3 (15.9)	66.0 (14.2)	67.2 (13.6)	62.4 (11.8)	64.8 (14.7)	0.15
Female No. (%)	13 (65)	19 (44)	29 (39)	17 (34)	24 (32)	15 (30)	23 (27)	15 (32)	155 (35)	0.61
Charlson Comorbidity Index Mean (SD)	1.2 (0.9)	2.0 (1.6)	2.9 (2.1)	3.0 (2.3)	3.0 (2.2)	3.8 (2.5)	3.6 (2.2)	3.5 (1.8)	3.1 (2.2)	<0.001
SAPS II Mean (SD)	29.6 (17.0)	30.6 (15.5)	35.1 (17.4)	31.8 (16.6)	32.9 (13.7)	33.7 (13.1)	34.4 (14.2)	36.2 (17.3)	33.5 (15.4)	0.53
C-reactive protein Day 0(ug/mL) Mean (SD)	22.2 (24.6)	52.1 (51)	101.9 (69.4)	120.0 (80.5)	129.6 (74.9)	155.2 (84)	164.5 (94.1)	167.7 (103.8)	125.2 (89.4)	<0.001
Day 0 25(OH)D(ng/mL) Mean (SD)	14.4 (4.9)	14.4 (5.1)	15.2 (6.8)	14.1 (6.9)	12.4 (4.2)	13.8 (6.1)	14.3 (16.2)	12.5 (4.8)	13.9 (8.7)	0.62
Vitamin D_3 Intervention No. (%)	9 (45)	22 (51)	31 (42)	21 (42)	43 (57)	21 (42)	43 (51)	29 (62)	219 (49)	0.26
Change in 25(OH)D Day 0 to Day 3(ng/mL) Median [IQR]	3.4 [-0.4, 21.3]	3.8 [0.1, 38.1]	3.0 [0, 24.1]	2.2 [-1.2, 16.1]	5.4 [0.6, 16.7]	1.8 [-0.7, 10.9]	2.5 [0.1, 10.8]	4.2 [-0.4, 10.1]	3.3 [0, 16.7]	<0.001
Total Bilirubin Day 0(mg/dL) Mean (SD)	0.5 (0.3)	0.6 (0.4)	0.7 (0.7)	1.1 (1.4)	1.1 (1.1)	1.7 (2.0)	2.4 (2.9)	3.7 (5.8)	1.6 (2.6)	<0.001
Creatinine Day 0(mg/dL) Mean (SD)	0.7 (0.2)	0.8 (0.2)	1.1 (0.8)	1.2 (0.7)	1.4 (1.0)	1.7 (0.9)	1.8 (1.2)	2.2 (1.2)	1.4 (1.0)	<0.001
ICU										
Anesthesia ICU No. (%)	2 (10)	8 (19)	11 (15)	8 (16)	19 (25)	10 (20)	21 (25)	4 (9)	83 (19)	
Cardiac Surgery ICU No. (%)	0 (0)	3 (7)	14 (19)	11 (22)	23 (31)	22 (44)	35 (41)	22 (47)	130 (29)	
Surgical ICU No. (%)	1 (5)	0 (0)	4 (5)	4 (8)	6 (8)	4 (8)	4 (5)	0 (0)	23 (5)	
Medical ICU No. (%)	1 (5)	4 (9)	12 (16)	13 (26)	19 (25)	12 (24)	20 (24)	18 (38)	99 (22)	
Neurological ICU No. (%)	16 (80)	28 (65)	33 (45)	14 (28)	8 (11)	2 (4)	5 (6)	3 (6)	109 (25)	

Supplemental Table 1. Additional Cohort Characteristics (Continued)

Characteristic	Procalcitonin at Day 0								Total	P-value
	0.00 - <0.05 ug/L	0.05 - <0.10 ug/L	0.10 - <0.25 ug/L	0.25 - <0.50 ug/L	0.50 - <1.00 ug/L	1.00 - <2.00 ug/L	2.00 - <10.00 ug/L	≥10.00 ug/L		
Admission Diagnosis										
Brain Surgery No. (%)	0 (0)	1 (2)	1 (1)	1 (2)	0 (0)	0 (0)	1 (1)	0 (0)	4 (1)	
Cardiac surgery No. (%)	0 (0)	2 (5)	9 (12)	9 (18)	17 (23)	12 (24)	24 (28)	12 (26)	85 (19)	
Cardiovascular No. (%)	1 (5)	1 (3)	9 (12)	9 (18)	10 (13)	11 (22)	12 (14)	4 (9)	57 (13)	
Gastrointestinal/liver No. (%)	1 (5)	0 (0)	0 (0)	1 (2)	5 (7)	3 (6)	3 (6)	1 (2)	14 (3)	
Hematologic/oncologic No. (%)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	1 (2)	2 (0)	
Metabolic No. (%)	0 (0)	0 (0)	0 (0)	1 (2)	1 (1)	0 (0)	0 (0)	1 (2)	3 (1)	
Neurologic No. (%)	16 (80)	29 (67)	34 (46)	11 (22)	7 (9)	2 (4)	6 (7)	2 (4)	107 (24)	
Other non-operative	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	1 (1)	1 (2)	3 (1)	
Other operative No. (%)	1 (5)	1 (2)	1 (1)	2 (4)	3 (4)	1 (2)	3 (4)	1 (2)	13 (3)	
Renal No. (%)	0 (0)	0 (0)	0 (0)	0 (0)	2 (3)	2 (4)	0 (0)	2 (4)	6 (1)	
Respiratory No. (%)	0 (0)	3 (7)	7 (9)	7 (14)	10 (13)	3 (6)	9 (11)	4 (9)	43 (10)	
Sepsis/infectious No. (%)	0 (0)	1 (2)	3 (4)	5 (10)	5 (7)	3 (6)	9 (11)	10 (21)	36 (8)	
Thoracic surgery No. (%)	0 (0)	0 (0)	3 (4)	0 (0)	2 (3)	3 (6)	4 (5)	1 (2)	13 (3)	
Transplantation No. (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4)	4 (5)	6 (13)	12 (3)	
Trauma No. (%)	1 (5)	5 (12)	5 (7)	4 (8)	10 (13)	5 (10)	6 (7)	1 (2)	37 (8)	
Vascular surgery No. (%)	0 (0)	0 (0)	1 (1)	0 (0)	2 (3)	3 (6)	3 (4)	0 (0)	9 (2)	
28-day mortality No. (%)	1 (5)	5 (12)	15 (20)	9 (18)	15 (20)	13 (26)	33 (39)	23 (49)	114 (26)	<0.001

Supplemental Table 2. At randomization (Day 0) OPLS-DA model goodness of fit and predictive ability

OPLS-DA			Permutation (N=200)		CV-ANOVA
R2X	R2Y	Q2	R2 intercept (*x*-axis, *y*-axis)	Q2 intercept (*x*-axis, *y*-axis)	P-value
0.117	1.00	0.422	0.00, 0.156	0.00, -0.187	<0.001

Supplemental Table 3. Metabolites significantly increased with increased Procalcitonin over days 0-7

Metabolite	β Coefficient	P-value	FDR adjusted P-value	-log$_{10}$p	Super Pathway	Sub Pathway
N-acetyl-aspartyl-glutamate (NAAG)	1.70E-03	1.04E-02	3.20E-02	1.98	Amino Acid	Glutamate Metabolism
carboxyethyl-GABA	2.13E-03	5.54E-05	4.93E-04	4.26	Amino Acid	Glutamate Metabolism
citramalate	2.52E-03	2.63E-04	1.61E-03	3.58	Amino Acid	Glutamate Metabolism
alpha-ketoglutaramate*	3.37E-03	4.32E-10	2.85E-08	9.36	Amino Acid	Glutamate Metabolism
cysteinylglycine	3.03E-03	5.58E-04	3.14E-03	3.25	Amino Acid	Glutathione Metabolism
N-acetylthreonine	2.17E-03	4.76E-05	4.42E-04	4.32	Amino Acid	Glycine, Serine and Threonine Metabolism
N-acetylserine	2.29E-03	1.27E-05	1.53E-04	4.89	Amino Acid	Glycine, Serine and Threonine Metabolism
1-methyl-4-imidazoleacetate	1.57E-03	7.71E-03	2.65E-02	2.11	Amino Acid	Histidine Metabolism
N-acetylcarnosine	1.73E-03	8.47E-03	2.82E-02	2.07	Amino Acid	Histidine Metabolism
1-methylhistidine	1.95E-03	4.11E-03	1.59E-02	2.39	Amino Acid	Histidine Metabolism
N-acetylhistidine	2.18E-03	2.00E-03	8.84E-03	2.70	Amino Acid	Histidine Metabolism
1-ribosyl-imidazoleacetate*	2.23E-03	1.11E-04	8.19E-04	3.96	Amino Acid	Histidine Metabolism
imidazole lactate	2.33E-03	1.39E-04	9.44E-04	3.86	Amino Acid	Histidine Metabolism
formiminoglutamate	2.63E-03	7.76E-04	4.16E-03	3.11	Amino Acid	Histidine Metabolism
N-acetylvaline	1.53E-03	3.75E-03	1.48E-02	2.43	Amino Acid	BCAA Metabolism
ethylmalonate	1.55E-03	8.44E-03	2.82E-02	2.07	Amino Acid	BCAA Metabolism
beta-hydroxyisovalerate	1.75E-03	2.87E-03	1.20E-02	2.54	Amino Acid	BCAA Metabolism
3-methylglutaconate	1.91E-03	2.08E-03	9.09E-03	2.68	Amino Acid	BCAA Metabolism
3-hydroxy-2-ethylpropionate	2.13E-03	3.24E-04	1.94E-03	3.49	Amino Acid	BCAA Metabolism
2,3-dihydroxy-2-methylbutyrate	3.67E-03	4.16E-07	1.10E-05	6.38	Amino Acid	BCAA Metabolism
methylsuccinate	4.13E-03	1.56E-09	8.58E-08	8.81	Amino Acid	BCAA Metabolism
5-(galactosylhydroxy)-L-lysine	1.68E-03	9.95E-03	3.12E-02	2.00	Amino Acid	Lysine Metabolism
N6,N6,N6-trimethyllysine	2.68E-03	2.06E-05	2.23E-04	4.69	Amino Acid	Lysine Metabolism
cysteine	1.95E-03	1.56E-03	7.22E-03	2.81	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
N-acetyltaurine	2.09E-03	3.40E-04	2.00E-03	3.47	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism

Supplemental Table 3. Metabolites significantly increased with increased Procalcitonin over days 0-7 (Continued)

Metabolite	β Coefficient	P-value	FDR adjusted P-value	-log$_{10}$p	Super Pathway	Sub Pathway
S-adenosylhomocysteine (SAH)	2.41E-03	2.76E-04	1.67E-03	3.56	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
5-methylthioribose	4.49E-03	1.01E-08	3.99E-07	8.00	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
cystathionine	5.02E-03	3.73E-11	4.10E-09	10.43	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
N-acetylphenylalanine	1.97E-03	9.15E-03	2.94E-02	2.04	Amino Acid	Phenylalanine Metabolism
4-hydroxyphenylacetate	2.92E-03	3.58E-03	1.45E-02	2.45	Amino Acid	Phenylalanine Metabolism
phenyllactate (PLA)	2.97E-03	1.13E-05	1.40E-04	4.95	Amino Acid	Phenylalanine Metabolism
N-acetylputrescine	2.25E-03	8.00E-04	4.25E-03	3.10	Amino Acid	Polyamine Metabolism
N-acetyl-isoputreanine*	2.31E-03	3.56E-04	2.06E-03	3.45	Amino Acid	Polyamine Metabolism
4-acetamidobutanoate	2.86E-03	4.07E-06	6.65E-05	5.39	Amino Acid	Polyamine Metabolism
(N(1) + N(8))-acetylspermidine	3.52E-03	1.59E-06	3.00E-05	5.80	Amino Acid	Polyamine Metabolism
acisoga	3.84E-03	6.20E-07	1.46E-05	6.21	Amino Acid	Polyamine Metabolism
N1,N12-diacetylspermine	6.03E-03	7.60E-16	5.01E-13	15.12	Amino Acid	Polyamine Metabolism
indoleacetate	2.16E-03	9.40E-03	2.98E-02	2.03	Amino Acid	Tryptophan Metabolism
indole-3-carboxylate	2.32E-03	6.23E-04	3.45E-03	3.21	Amino Acid	Tryptophan Metabolism
kynurenine	2.56E-03	8.47E-07	1.86E-05	6.07	Amino Acid	Tryptophan Metabolism
indoleacetylglutamine	2.85E-03	1.16E-03	5.81E-03	2.93	Amino Acid	Tryptophan Metabolism
picolinate	2.86E-03	1.69E-04	1.11E-03	3.77	Amino Acid	Tryptophan Metabolism
3-indoxyl sulfate	3.58E-03	8.52E-06	1.15E-04	5.07	Amino Acid	Tryptophan Metabolism
xanthurenate	4.22E-03	1.83E-06	3.35E-05	5.74	Amino Acid	Tryptophan Metabolism
kynurenate	4.88E-03	9.08E-12	1.20E-09	11.04	Amino Acid	Tryptophan Metabolism
N-formylanthranilic acid	5.11E-03	3.03E-14	9.97E-12	13.52	Amino Acid	Tryptophan Metabolism
N-acetylkynurenine (2)	5.24E-03	1.94E-08	7.11E-07	7.71	Amino Acid	Tryptophan Metabolism
N-acetyltryptophan	1.48E-02	5.56E-10	3.33E-08	9.25	Amino Acid	Tryptophan Metabolism
vanillactate	2.03E-03	1.51E-03	7.10E-03	2.82	Amino Acid	Tyrosine Metabolism
3-(4-hydroxyphenyl)lactate (HPLA)	2.27E-03	1.49E-04	9.92E-04	3.83	Amino Acid	Tyrosine Metabolism

Supplemental Table 3. Metabolites significantly increased with increased Procalcitonin over days 0-7 (Continued)

Metabolite	β Coefficient	P-value	FDR adjusted P-value	-log$_{10}$p	Super Pathway	Sub Pathway
phenol sulfate	2.71E-03	5.61E-03	2.04E-02	2.25	Amino Acid	Tyrosine Metabolism
homovanillate sulfate	3.89E-03	5.15E-06	7.72E-05	5.29	Amino Acid	Tyrosine Metabolism
homovanillate (HVA)	3.92E-03	6.30E-08	1.98E-06	7.20	Amino Acid	Tyrosine Metabolism
4-methoxyphenol sulfate	4.08E-03	4.45E-06	6.82E-05	5.35	Amino Acid	Tyrosine Metabolism
vanillylmandelate (VMA)	4.96E-03	6.87E-11	6.47E-09	10.16	Amino Acid	Tyrosine Metabolism
2-hydroxyphenylacetate	5.29E-03	1.32E-10	1.09E-08	9.88	Amino Acid	Tyrosine Metabolism
urea	1.65E-03	7.62E-03	2.63E-02	2.12	Amino Acid	Urea cycle; Arginine and Proline Metabolism
2-oxoarginine*	2.03E-03	6.09E-03	2.16E-02	2.22	Amino Acid	Urea cycle; Arginine and Proline Metabolism
N2,N5-diacetylornithine	2.73E-03	1.34E-03	6.44E-03	2.87	Amino Acid	Urea cycle; Arginine and Proline Metabolism
glucuronate	2.00E-03	1.27E-02	3.85E-02	1.90	Carbohydrate	Aminosugar Metabolism
N-acetylneuraminate	2.20E-03	4.61E-05	4.34E-04	4.34	Carbohydrate	Aminosugar Metabolism
erythronate*	2.20E-03	2.78E-05	2.90E-04	4.56	Carbohydrate	Aminosugar Metabolism
mannitol/sorbitol	3.95E-03	1.30E-03	6.32E-03	2.88	Carbohydrate	Fructose, Mannose and Galactose Metabolism
maltotetraose	3.08E-03	1.74E-03	7.85E-03	2.76	Carbohydrate	Glycogen Metabolism
maltose	4.10E-03	1.37E-04	9.44E-04	3.86	Carbohydrate	Glycogen Metabolism
arabinose	2.48E-03	8.07E-04	4.26E-03	3.09	Carbohydrate	Pentose Metabolism
sedoheptulose	2.89E-03	5.21E-05	4.77E-04	4.28	Carbohydrate	Pentose Metabolism
gulonate*	2.43E-03	5.16E-03	1.90E-02	2.29	Cofactors and Vitamins	Ascorbate and Aldarate Metabolism
quinolinate	2.56E-03	7.58E-04	4.09E-03	3.12	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism
1-methylnicotinamide	3.16E-03	1.78E-04	1.15E-03	3.75	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism
nicotinamide riboside	4.73E-03	3.41E-10	2.49E-08	9.47	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism
2-methylcitrate/homocitrate	2.99E-03	1.27E-04	9.09E-04	3.90	Energy	TCA Cycle

Supplemental Table 3. Metabolites significantly increased with increased Procalcitonin over days 0-7 (Continued)

Metabolite	β Coefficient	P-value	FDR adjusted P-value	-log$_{10}$p	Super Pathway	Sub Pathway
androstenediol (3alpha, 17alpha) monosulfate (3)	1.76E-03	1.50E-02	4.39E-02	1.82	Lipid	Androgenic Steroids
epiandrosterone sulfate	1.91E-03	1.69E-02	4.71E-02	1.77	Lipid	Androgenic Steroids
andro steroid monosulfate C19H28O6S (1)*	2.54E-03	5.05E-03	1.87E-02	2.30	Lipid	Androgenic Steroids
5alpha-androstan-3alpha,17beta-diol monosulfate (1)	2.54E-03	8.34E-03	2.80E-02	2.08	Lipid	Androgenic Steroids
5alpha-androstan-3alpha,17beta-diol disulfate	3.42E-03	3.70E-05	3.54E-04	4.43	Lipid	Androgenic Steroids
hexanoylglutamine	2.30E-03	4.48E-03	1.70E-02	2.35	Lipid	Fatty Acid Metabolism (Acyl Glutamine)
3-hydroxybutyrylcarnitine (C4-OH)	1.80E-03	1.31E-02	3.88E-02	1.88	Lipid	Acylcarnitine
adipoylcarnitine (C6-DC)	2.17E-03	3.16E-03	1.30E-02	2.50	Lipid	Acylcarnitine
2-hydroxyphytanate*	2.20E-03	8.69E-04	4.54E-03	3.06	Lipid	Fatty Acid, Branched
adipate	1.88E-03	1.60E-02	4.55E-02	1.80	Lipid	Fatty Acid, Dicarboxylate
suberate (C8-DC)	2.02E-03	7.00E-03	2.44E-02	2.15	Lipid	Fatty Acid, Dicarboxylate
3-hydroxyadipate*	2.22E-03	8.93E-03	2.90E-02	2.05	Lipid	Fatty Acid, Dicarboxylate
heptenedioate (C7:1-DC)*	2.36E-03	3.78E-03	1.48E-02	2.42	Lipid	Fatty Acid, Dicarboxylate
octadecanedioate (C18)	2.49E-03	4.98E-03	1.86E-02	2.30	Lipid	Fatty Acid, Dicarboxylate
dodecenedioate (C12:1-DC)*	2.56E-03	3.57E-03	1.45E-02	2.45	Lipid	Fatty Acid, Dicarboxylate
octadecadienedioate (C18:2-DC)*	2.62E-03	9.02E-04	4.63E-03	3.04	Lipid	Fatty Acid, Dicarboxylate
octadecenedioate (C18:1-DC)*	2.63E-03	2.17E-03	9.43E-03	2.66	Lipid	Fatty Acid, Dicarboxylate
hexadecanedioate (C16)	3.17E-03	3.33E-04	1.98E-03	3.48	Lipid	Fatty Acid, Dicarboxylate
3-methyladipate	3.19E-03	3.15E-05	3.15E-04	4.50	Lipid	Fatty Acid, Dicarboxylate
dodecanedioate (C12)	3.23E-03	2.52E-04	1.55E-03	3.60	Lipid	Fatty Acid, Dicarboxylate
tetradecanedioate (C14)	3.85E-03	1.07E-05	1.36E-04	4.97	Lipid	Fatty Acid, Dicarboxylate
hexadecenedioate (C16:1-DC)*	4.23E-03	1.23E-06	2.47E-05	5.91	Lipid	Fatty Acid, Dicarboxylate
5-hydroxyhexanoate	2.43E-03	8.06E-05	6.25E-04	4.09	Lipid	Fatty Acid, Monohydroxy
3-hydroxyhexanoate	2.62E-03	2.00E-04	1.26E-03	3.70	Lipid	Fatty Acid, Monohydroxy
myo-inositol	4.59E-03	8.37E-09	3.68E-07	8.08	Lipid	Inositol Metabolism

Supplemental Table 3. Metabolites significantly increased with increased Procalcitonin over days 0-7 (Continued)

Metabolite	β Coefficient	P-value	FDR adjusted P-value	-log$_{10}$p	Super Pathway	Sub Pathway
10-undecenoate (11:1n1)	1.83E-03	1.65E-02	4.65E-02	1.78	Lipid	Medium Chain Fatty Acid
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	1.35E-03	1.53E-02	4.43E-02	1.81	Lipid	Phosphatidylethanolamine
1-palmitoyl-2-docosahexaenoyl-GPE (16:0/22:6)*	1.53E-03	2.70E-03	1.14E-02	2.57	Lipid	Phosphatidylethanolamine
1-oleoyl-2-docosahexaenoyl-GPE (18:1/22:6)*	1.74E-03	8.76E-03	2.87E-02	2.06	Lipid	Phosphatidylethanolamine
1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)*	1.81E-03	6.69E-04	3.68E-03	3.17	Lipid	Phosphatidylethanolamine
trimethylamine N-oxide	1.93E-03	1.65E-02	4.65E-02	1.78	Lipid	Phospholipid Metabolism
docosadienoate (22:2n6)	1.78E-03	1.15E-02	3.51E-02	1.94	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
pregnen-diol disulfate*	1.64E-03	1.25E-02	3.82E-02	1.90	Lipid	Pregnenolone Steroids
21-hydroxypregnenolone disulfate	2.36E-03	1.57E-03	7.22E-03	2.80	Lipid	Pregnenolone Steroids
taurochenodeoxycholate	2.69E-03	8.55E-03	2.83E-02	2.07	Lipid	Primary Bile Acid Metabolism
5alpha-pregnan-3beta,20alpha-diol monosulfate (2)	1.95E-03	1.28E-02	3.85E-02	1.89	Lipid	Progestin Steroids
5alpha-pregnan-3beta,20alpha-diol disulfate	3.17E-03	3.71E-05	3.54E-04	4.43	Lipid	Progestin Steroids
5alpha-pregnan-3beta,20beta-diol monosulfate (1)	3.65E-03	2.05E-05	2.23E-04	4.69	Lipid	Progestin Steroids
pregnanediol-3-glucuronide	4.26E-03	1.15E-07	3.30E-06	6.94	Lipid	Progestin Steroids
5alpha-pregnan-diol disulfate	5.51E-03	8.20E-09	3.68E-07	8.09	Lipid	Progestin Steroids
glycolithocholate sulfate*	2.48E-03	1.57E-02	4.49E-02	1.80	Lipid	Secondary Bile Acid Metabolism
glycodeoxycholate sulfate	3.20E-03	4.15E-03	1.60E-02	2.38	Lipid	Secondary Bile Acid Metabolism
taurolithocholate 3-sulfate	3.62E-03	3.78E-04	2.17E-03	3.42	Lipid	Secondary Bile Acid Metabolism
N1-methylinosine	1.70E-03	3.90E-03	1.52E-02	2.41	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing
xanthosine	3.21E-03	6.44E-05	5.44E-04	4.19	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing
N6-carbamoylthreonyladenosine	1.92E-03	1.63E-03	7.48E-03	2.79	Nucleotide	Purine Metabolism, Adenine containing
N6-succinyladenosine	4.76E-03	4.16E-12	6.85E-10	11.38	Nucleotide	Purine Metabolism, Adenine containing

Supplemental Table 3. Metabolites significantly increased with increased Procalcitonin over days 0-7 (Continued)

Metabolite	β Coefficient	P-value	FDR adjusted P-value	-log$_{10}$p	Super Pathway	Sub Pathway
N2,N2-dimethylguanosine	2.73E-03	8.27E-07	1.86E-05	6.08	Nucleotide	Purine Metabolism, Guanine containing
pseudouridine	1.38E-03	5.69E-03	2.05E-02	2.25	Nucleotide	Pyrimidine Metabolism, Uracil containing
5,6-dihydrouridine	1.61E-03	3.72E-03	1.48E-02	2.43	Nucleotide	Pyrimidine Metabolism, Uracil containing
N-acetyl-beta-alanine	3.70E-03	2.14E-12	4.71E-10	11.67	Nucleotide	Pyrimidine Metabolism, Uracil containing
glucuronide of C10H18O2 (7)*	3.36E-03	2.65E-03	1.13E-02	2.58	Partially Characterized Molecules	Partially Characterized Molecules
glycine conjugate of C10H14O2 (1)*	3.54E-03	1.58E-05	1.82E-04	4.80	Partially Characterized Molecules	Partially Characterized Molecules
phenylacetylglutamine	2.82E-03	3.48E-04	2.03E-03	3.46	Peptide	Acetylated Peptides
phenylacetylmethionine	2.93E-03	9.81E-04	4.97E-03	3.01	Peptide	Acetylated Peptides
phenylacetylglutamate	4.02E-03	3.01E-05	3.10E-04	4.52	Peptide	Acetylated Peptides
4-vinylphenol sulfate	2.40E-03	1.65E-02	4.65E-02	1.78	Xenobiotics	Benzoate Metabolism
4-hydroxyhippurate	2.88E-03	2.01E-03	8.84E-03	2.70	Xenobiotics	Benzoate Metabolism
catechol sulfate	2.94E-03	1.07E-02	3.31E-02	1.97	Xenobiotics	Benzoate Metabolism
3-methyl catechol sulfate (1)	3.51E-03	1.66E-03	7.52E-03	2.78	Xenobiotics	Benzoate Metabolism
4-methylguaiacol sulfate	4.35E-03	7.33E-06	1.03E-04	5.13	Xenobiotics	Benzoate Metabolism
4-methylcatechol sulfate	4.81E-03	2.09E-06	3.63E-05	5.68	Xenobiotics	Benzoate Metabolism
hippurate	5.04E-03	1.05E-06	2.23E-05	5.98	Xenobiotics	Benzoate Metabolism
guaiacol sulfate	5.69E-03	3.86E-08	1.27E-06	7.41	Xenobiotics	Benzoate Metabolism
succinimide	1.54E-03	1.52E-02	4.41E-02	1.82	Xenobiotics	Chemical
O-sulfo-L-tyrosine	1.73E-03	3.12E-03	1.29E-02	2.51	Xenobiotics	Chemical
6-hydroxyindole sulfate	3.24E-03	7.27E-05	6.07E-04	4.14	Xenobiotics	Chemical
trizma acetate	4.93E-03	2.01E-04	1.26E-03	3.70	Xenobiotics	Chemical
4-acetamidophenylglucuronide	4.94E-03	8.66E-03	2.85E-02	2.06	Xenobiotics	Drug - Analgesics, Anesthetics
furosemide	5.50E-03	2.43E-05	2.58E-04	4.61	Xenobiotics	Drug - Cardiovascular

Supplemental Table 3. Metabolites significantly increased with increased Procalcitonin over days 0-7 (Continued)

Metabolite	β Coefficient	P-value	FDR adjusted P-value	-log$_{10}$p	Super Pathway	Sub Pathway
pantoprazole	4.94E-03	7.20E-06	1.03E-04	5.14	Xenobiotics	Drug - Gastrointestinal
hydroquinone sulfate	4.21E-03	4.14E-06	6.65E-05	5.38	Xenobiotics	Drug - Topical Agents
2-keto-3-deoxy-gluconate	1.81E-03	7.81E-03	2.67E-02	2.11	Xenobiotics	Food Component/Plant
phytanate	1.99E-03	1.43E-02	4.23E-02	1.84	Xenobiotics	Food Component/Plant
erythritol	2.20E-03	8.27E-05	6.34E-04	4.08	Xenobiotics	Food Component/Plant
2,3-dihydroxyisovalerate	2.76E-03	3.68E-03	1.47E-02	2.43	Xenobiotics	Food Component/Plant
cinnamoylglycine	3.02E-03	4.84E-04	2.75E-03	3.31	Xenobiotics	Food Component/Plant
indolin-2-one	3.88E-03	1.23E-07	3.37E-06	6.91	Xenobiotics	Food Component/Plant
gluconate	5.34E-03	1.38E-04	9.44E-04	3.86	Xenobiotics	Food Component/Plant
7-methylurate	2.17E-03	1.78E-03	7.93E-03	2.75	Xenobiotics	Xanthine Metabolism
1,3,7-trimethylurate	2.50E-03	5.02E-03	1.87E-02	2.30	Xenobiotics	Xanthine Metabolism
1,7-dimethylurate	2.59E-03	8.93E-03	2.90E-02	2.05	Xenobiotics	Xanthine Metabolism
1-methylurate	3.24E-03	3.28E-05	3.23E-04	4.48	Xenobiotics	Xanthine Metabolism

Note: Using repeated measures data (day 0, 3 and 7), the association between relative quantitation of each individual metabolite noted above and Procalcitonin levels over time were determined utilizing linear mixed-effects models correcting for age, sex, baseline 25(OH)D, absolute increase in 25(OH)D, Simplified Acute Physiology Score (SAPS) II, plasma day, admission diagnosis and individual subjects (as the random-intercept). All significant mixed-effects associations have Benjamini-Hochberg adjusted P-value <0.05. BCAA is Branched-Chain Amino Acids inclusive of Leucine, Isoleucine and Valine. For the Acylcarnitines sub pathway: a capital C is followed by the number of carbons within the fatty acyl group attached to the carnitine. A colon followed by a number is one or more unsaturated carbons in the acylcarnitine ester (i.e. C10:1 is a monounsaturated C10 acylcarnitine). OH following the carbon number is a hydroxylic acylcarnitine. DC following the carbon number is a dicarboxylic acylcarnitine. GPE is glycerophosphoethanolamine.

Supplemental Table 4. Metabolites significantly decreased with increased Procalcitonin over days 0-7

Metabolite	β Coefficient	P-value	FDR adjusted P-value	-log$_{10}$p	Super Pathway	Sub Pathway
4-hydroxyglutamate	-1.92E-03	1.31E-02	3.88E-02	1.88	Amino Acid	Glutamate Metabolism
glutamate	-3.25E-03	7.42E-08	2.22E-06	7.13	Amino Acid	Glutamate Metabolism
2-aminobutyrate	-1.59E-03	1.01E-02	3.16E-02	1.99	Amino Acid	Glutathione Metabolism
dimethylglycine	-1.69E-03	3.66E-03	1.47E-02	2.44	Amino Acid	Glycine, Serine and Threonine Metabolism
cystine	-1.85E-03	8.05E-03	2.74E-02	2.09	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
N-acetylmethionine	-1.90E-03	1.26E-02	3.84E-02	1.90	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
S-methylcysteine sulfoxide	-2.04E-03	3.02E-03	1.26E-02	2.52	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
taurine	-2.53E-03	1.14E-04	8.33E-04	3.94	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
S-methylcysteine	-2.79E-03	5.54E-07	1.35E-05	6.26	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
hypotaurine	-2.81E-03	1.29E-03	6.32E-03	2.89	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
tryptophan betaine	-1.11E-03	7.16E-03	2.48E-02	2.14	Amino Acid	Tryptophan Metabolism
thyroxine	-1.73E-03	7.14E-04	3.89E-03	3.15	Amino Acid	Tyrosine Metabolism
N-methylhydroxyproline	-5.15E-03	5.79E-05	5.09E-04	4.24	Amino Acid	Urea cycle; Arginine and Proline Metabolism
fructose	-1.77E-03	5.20E-03	1.90E-02	2.28	Carbohydrate	Fructose, Mannose and Galactose Metabolism
nicotinamide	-3.34E-03	2.05E-04	1.28E-03	3.69	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism
pantothenate (Vitamin B5)	-1.51E-03	4.66E-03	1.75E-02	2.33	Cofactors and Vitamins	Pantothenate and CoA Metabolism
beta-cryptoxanthin	-1.26E-03	4.30E-03	1.65E-02	2.37	Cofactors and Vitamins	Vitamin A Metabolism
carotene diol (3)	-1.50E-03	1.54E-02	4.43E-02	1.81	Cofactors and Vitamins	Vitamin A Metabolism
sphingomyelin (d18:0/18:0, d19:0/17:0)*	-1.83E-03	9.23E-03	2.95E-02	2.03	Lipid	Dihydrosphingomyelins
behenoyl dihydrosphingomyelin (d18:0/22:0)*	-2.18E-03	6.16E-04	3.44E-03	3.21	Lipid	Dihydrosphingomyelins

Supplemental Table 4. Metabolites significantly decreased with increased Procalcitonin over days 0-7 (Continued)

Metabolite	β Coefficient	P-value	FDR adjusted P-value	-log$_{10}$p	Super Pathway	Sub Pathway
sphingomyelin (d18:0/18:0, d19:0/17:0)*	-1.83E-03	9.23E-03	2.95E-02	2.03	Lipid	Dihydrosphingomyelins
behenoyl dihydrosphingomyelin (d18:0/22:0)*	-2.18E-03	6.16E-04	3.44E-03	3.21	Lipid	Dihydrosphingomyelins
sphingomyelin (d18:0/20:0, d16:0/22:0)*	-2.42E-03	1.87E-04	1.20E-03	3.73	Lipid	Dihydrosphingomyelins
linoleoylcholine*	-3.37E-03	1.47E-04	9.91E-04	3.83	Lipid	Acyl Choline
dihomo-linolenoyl-choline	-3.43E-03	1.32E-04	9.33E-04	3.88	Lipid	Acyl Choline
docosahexaenoylcholine	-3.45E-03	5.45E-05	4.92E-04	4.26	Lipid	Acyl Choline
palmitoylcholine	-3.46E-03	7.56E-05	6.15E-04	4.12	Lipid	Acyl Choline
oleoylcholine	-3.54E-03	7.36E-05	6.07E-04	4.13	Lipid	Acyl Choline
stearoylcholine*	-3.60E-03	1.26E-04	9.09E-04	3.90	Lipid	Acyl Choline
arachidonoylcholine	-3.91E-03	4.38E-06	6.82E-05	5.36	Lipid	Acyl Choline
oleoylcarnitine (C18)	-1.58E-03	1.68E-02	4.70E-02	1.78	Lipid	Acylcarnitine
stearoylcarnitine (C18)	-1.85E-03	3.27E-03	1.34E-02	2.49	Lipid	Acylcarnitine
adrenoylcarnitine (C22:4)*	-1.95E-03	8.98E-03	2.90E-02	2.05	Lipid	Acylcarnitine
docosapentaenoylcarnitine (C22:5n3)*	-2.05E-03	1.02E-02	3.18E-02	1.99	Lipid	Acylcarnitine
dihomo-linoleoylcarnitine (C20:2)*	-2.11E-03	2.27E-03	9.77E-03	2.64	Lipid	Acylcarnitine
linoleoylcarnitine (C18:2)*	-2.20E-03	8.96E-04	4.63E-03	3.05	Lipid	Acylcarnitine
linolenoylcarnitine (C18:3)*	-2.32E-03	1.57E-03	7.22E-03	2.80	Lipid	Acylcarnitine
docosahexaenoylcarnitine (C22:6)*	-2.49E-03	1.44E-03	6.84E-03	2.84	Lipid	Acylcarnitine
lignoceroylcarnitine (C24)*	-2.59E-03	2.58E-06	4.36E-05	5.59	Lipid	Acylcarnitine
dihomo-linolenoylcarnitine (C20:3n3 or 6)*	-2.60E-03	1.76E-04	1.15E-03	3.75	Lipid	Acylcarnitine
arachidonoylcarnitine (C20:4)	-2.71E-03	1.34E-04	9.43E-04	3.87	Lipid	Acylcarnitine
cerotoylcarnitine (C26)*	-2.88E-03	5.01E-07	1.27E-05	6.30	Lipid	Acylcarnitine
ximenoylcarnitine (C26:1)*	-3.49E-03	1.03E-08	3.99E-07	7.99	Lipid	Acylcarnitine
2-aminoheptanoate	-1.93E-03	6.01E-03	2.14E-02	2.22	Lipid	Fatty Acid, Amino
2-aminooctanoate	-3.41E-03	1.15E-05	1.40E-04	4.94	Lipid	Fatty Acid, Amino

Supplemental Table 4. Metabolites significantly decreased with increased Procalcitonin over days 0-7 (Continued)

Metabolite	β Coefficient	P-value	FDR adjusted P-value	-log$_{10}$p	Super Pathway	Sub Pathway
glycosyl ceramide (d18:1/20:0, d16:1/22:0)*	-1.25E-03	1.44E-02	4.24E-02	1.84	Lipid	Hexosylceramides
glycosyl-N-(2-hydroxynervonoyl)-sphingosine (d18:1/24:1(2OH))*	-1.96E-03	1.75E-03	7.85E-03	2.76	Lipid	Hexosylceramides
1-palmitoyl-GPA (16:0)	-1.97E-03	9.25E-03	2.95E-02	2.03	Lipid	Lysophospholipid
1-palmitoyl-GPI* (16:0)	-1.98E-03	6.76E-03	2.37E-02	2.17	Lipid	Lysophospholipid
1-linolenoyl-GPC (18:3)*	-2.32E-03	1.30E-03	6.32E-03	2.89	Lipid	Lysophospholipid
1-palmitoleoyl-GPC* (16:1)*	-2.60E-03	1.41E-05	1.66E-04	4.85	Lipid	Lysophospholipid
1-arachidonoyl-GPC* (20:4)*	-2.61E-03	1.34E-06	2.60E-05	5.87	Lipid	Lysophospholipid
2-palmitoyl-GPC* (16:0)*	-2.78E-03	9.30E-06	1.20E-04	5.03	Lipid	Lysophospholipid
1-lignoceroyl-GPC (24:0)	-4.03E-03	7.68E-09	3.68E-07	8.11	Lipid	Lysophospholipid
1-(1-enyl-palmitoyl)-GPE (P-16:0)*	-2.79E-03	6.21E-05	5.31E-04	4.21	Lipid	Lysoplasmalogen
1-(1-enyl-stearoyl)-GPE (P-18:0)*	-3.02E-03	1.67E-05	1.90E-04	4.78	Lipid	Lysoplasmalogen
1-(1-enyl-oleoyl)-GPE (P-18:1)*	-3.09E-03	3.09E-05	3.13E-04	4.51	Lipid	Lysoplasmalogen
1-(1-enyl-palmitoyl)-GPC (P-16:0)*	-3.45E-03	2.88E-08	9.99E-07	7.54	Lipid	Lysoplasmalogen
phosphoethanolamine	-2.09E-03	6.23E-03	2.19E-02	2.21	Lipid	Phospholipid Metabolism
glycerophosphorylcholine (GPC)	-3.13E-03	1.98E-06	3.53E-05	5.70	Lipid	Phospholipid Metabolism
1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4)*	-1.69E-03	5.66E-03	2.05E-02	2.25	Lipid	Plasmalogen
1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (P-16:0/18:2)*	-1.80E-03	5.97E-03	2.14E-02	2.22	Lipid	Plasmalogen
1-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2)*	-2.33E-03	6.08E-05	5.27E-04	4.22	Lipid	Plasmalogen
1-(1-enyl-stearoyl)-2-oleoyl-GPE (P-18:0/18:1)	-2.42E-03	9.88E-05	7.49E-04	4.01	Lipid	Plasmalogen
ursodeoxycholate	-3.74E-03	1.37E-03	6.55E-03	2.86	Lipid	Secondary Bile Acid Metabolism
sphingomyelin (d17:1/14:0, d16:1/15:0)*	-1.28E-03	1.30E-02	3.88E-02	1.89	Lipid	Sphingomyelins
sphingomyelin (d17:2/16:0, d18:2/15:0)*	-1.32E-03	9.54E-03	3.01E-02	2.02	Lipid	Sphingomyelins
sphingomyelin (d18:2/21:0, d16:2/23:0)*	-1.45E-03	2.42E-03	1.04E-02	2.62	Lipid	Sphingomyelins

Supplemental Table 4. Metabolites significantly decreased with increased Procalcitonin over days 0-7 (Continued)

Metabolite	β Coefficient	P-value	FDR adjusted P-value	-log$_{10}$p	Super Pathway	Sub Pathway
sphingomyelin (d18:1/19:0, d19:1/18:0)*	-1.55E-03	1.19E-03	5.89E-03	2.92	Lipid	Sphingomyelins
sphingomyelin (d18:2/14:0, d18:1/14:1)*	-1.60E-03	1.09E-03	5.48E-03	2.96	Lipid	Sphingomyelins
sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	-1.96E-03	7.92E-05	6.21E-04	4.10	Lipid	Sphingomyelins
sphingomyelin (d18:1/25:0, d19:0/24:1, d20:1/23:0, d19:1/24:0)*	-1.96E-03	1.09E-04	8.17E-04	3.96	Lipid	Sphingomyelins
sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)*	-1.97E-03	7.70E-05	6.18E-04	4.11	Lipid	Sphingomyelins
lignoceroyl sphingomyelin (d18:1/24:0)	-2.23E-03	8.86E-06	1.17E-04	5.05	Lipid	Sphingomyelins
tricosanoyl sphingomyelin (d18:1/23:0)*	-2.25E-03	6.40E-06	9.37E-05	5.19	Lipid	Sphingomyelins
hexadecasphingosine (d16:1)*	-2.63E-03	9.06E-04	4.63E-03	3.04	Lipid	Sphingosines
ADP	-2.44E-03	1.51E-02	4.39E-02	1.82	Nucleotide	Purine Metabolism, Adenine containing
uridine	-2.82E-03	1.08E-06	2.23E-05	5.97	Nucleotide	Pyrimidine Metabolism, Uracil containing
gamma-glutamylthreonine	-1.94E-03	4.36E-03	1.66E-02	2.36	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamylcitrulline*	-3.19E-03	1.78E-05	1.99E-04	4.75	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamylglutamate	-3.43E-03	8.05E-06	1.11E-04	5.09	Peptide	Gamma-glutamyl Amino Acid
thioproline	-1.59E-03	8.13E-03	2.75E-02	2.09	Xenobiotics	Chemical
ergothioneine	-2.01E-03	7.85E-05	6.21E-04	4.11	Xenobiotics	Food Component/Plant

Note: Using repeated measures data (day 0, 3 and 7), the association between relative quantitation of each individual metabolite noted above and Procalcitonin levels over time were determined utilizing linear mixed-effects models correcting for age, sex, baseline 25(OH)D, absolute increase in 25(OH)D, Simplified Acute Physiology Score (SAPS) II, plasma day, admission diagnosis and individual subjects (as the random-intercept). All significant mixed-effects associations have Benjamini-Hochberg adjusted P-value <0.05. BCAA is Branched-Chain Amino Acids inclusive of Leucine, Isoleucine and Valine. For the Acylcarnitines sub pathway: a capital C is followed by the number of carbons within the fatty acyl group attached to the carnitine. A colon followed by a number is one or more unsaturated carbons in the acylcarnitine ester (i.e. C10:1 is a monounsaturated C10 acylcarnitine). GPA is glycerophosphate; GPI is glycerophosphatidylinositol; GPC is glycerophosphorylcholine.

Supplemental Table 5. Day 0 PCT-specific Metabolic Networks with similar effects via Gaussian graphical models

Module	Module P- value	Metabolite	Super Pathway	Sub-pathway	Component P-value	Component β Coefficient
A	**6.62 E-17**	Beta-citrylglutamate	Amino Acid	Glutamate Metabolism	**3.06 E-66**	0.46
		Methylsuccinate	Amino Acid	BCAA Metabolism	**6.93 E-26**	0.30
		Ethylmalonate	Amino Acid	BCAA Metabolism	**2.16 E-40**	0.37
		Epiandrosterone sulfate	Lipid	Androgenic Steroids	5.29 E-01	-0.027
		5alpha-androstan-3beta,17beta-diol disulfate	Lipid	Androgenic Steroids	**2.03 E-14**	0.25
		Androsterone sulfate	Lipid	Androgenic Steroids	7.85 E-01	0.012
		5alpha-androstan-3alpha,17beta-diol monosulfate	Lipid	Androgenic Steroids	**2.26 E-05**	0.18
		5alpha-androstan-3alpha,17beta-diol disulfate	Lipid	Androgenic Steroids	**8.92 E-51**	0.59
		Adipate	Lipid	Fatty Acid, Dicarboxylate	**2.29 E-27**	0.35
		S-carboxymethyl-L-cysteine	Xenobiotics	Drug	**1.86 E-17**	0.31
B	**7.81 E-17**	1-ribosyl-imidazoleacetate	Amino Acid	Histidine Metabolism	**7.24 E-38**	0.37
		Imidazole lactate	Amino Acid	Histidine Metabolism	**7.03 E-63**	0.45
		1-methyl-4-imidazoleacetate	Amino Acid	Histidine Metabolism	**6.69 E-69**	0.50
		5-(galactosylhydroxy)-L-lysine	Amino Acid	Lysine Metabolism	**3.16 E-76**	0.53
		Gamma-glutamylhistidine	Peptide	Gamma-glutamyl Amino Acid	**2.08 E-08**	0.16
C	**1.12 E-21**	N-acetylputrescine	Amino Acid	Polyamine Metabolism	**7.65 E-34**	0.34
		4-acetamidobutanoate	Amino Acid	Polyamine Metabolism	**1.59 E-68**	0.53
		(N(1) + N(8))-acetylspermidine	Amino Acid	Polyamine Metabolism	**1.67 E-56**	0.47
		Acisoga	Amino Acid	Polyamine Metabolism	**4.49 E-50**	0.46
		N-acetyl-isoputreanine	Amino Acid	Polyamine Metabolism	**3.63 E-87**	0.60
		N-acetyl-beta-alanine	Nucleotide	Pyrimidine Metabolism	**3.83 E-84**	0.44
D	**1.58 E-20**	N-acetylkynurenine	Amino Acid	Tryptophan Metabolism	**1.46 E-75**	0.77
		Kynurenine	Amino Acid	Tryptophan Metabolism	**1.42 E-73**	0.40
		N-formylanthranilic acid	Amino Acid	Tryptophan Metabolism	**2.37 E-42**	0.39
		N-acetyltryptophan	Amino Acid	Tryptophan Metabolism	**3.34 E-68**	1.67
		Kynurenate	Amino Acid	Tryptophan Metabolism	**2.61 E-91**	0.80

Supplemental Table 5. Day 0 PCT-specific Metabolic Networks with similar effects via Gaussian graphical models

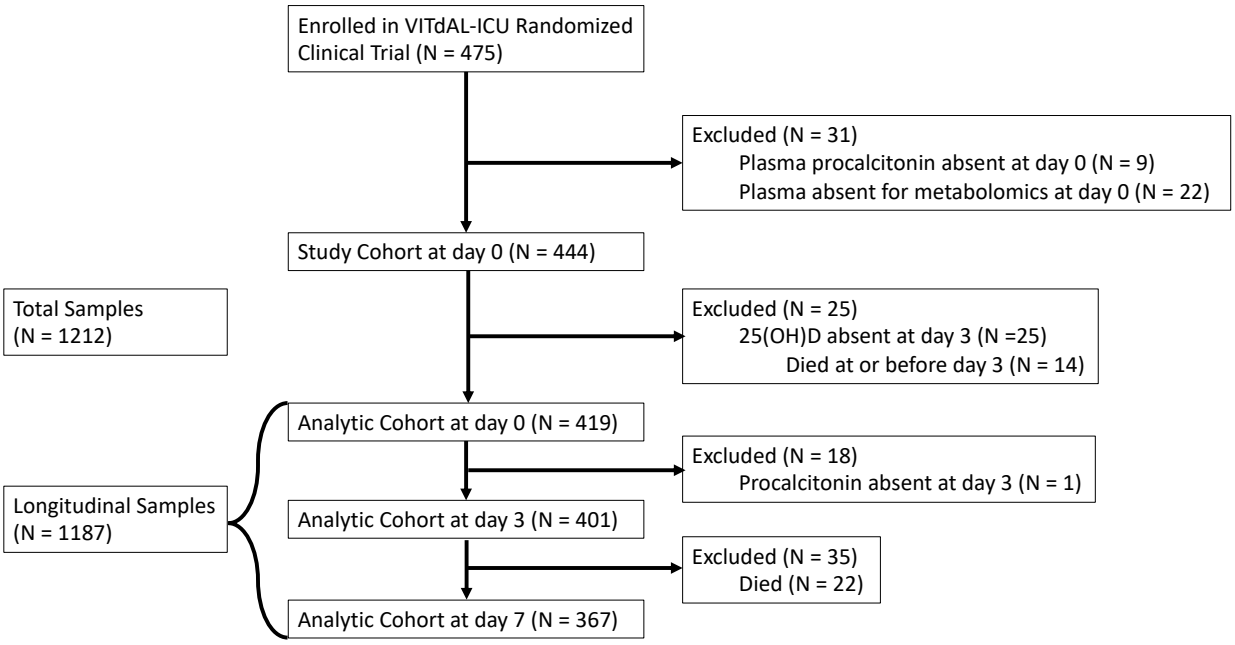
(Continued)

Module	Module P- value	Metabolite	Super Pathway	Sub-pathway	Component P-value	Component β Coefficient
D	**1.58 E-20**	Quinolinate	Cofactor	Nicotinate and Nicotinamide Metabolism	**6.34 E-97**	0.79
E	**2.76 E-08**	4-methoxyphenol sulfate	Amino Acid	Tyrosine Metabolism	**1.55 E-10**	0.24
		3-hydroxydecanoate	Lipid	Fatty Acid, Monohydroxy	**4.95 E-06**	0.14
		3-hydroxylaurate	Lipid	Fatty Acid, Monohydroxy	**5.07 E-06**	0.14
		Hydroquinone sulfate	Xenobiotics	Drug	**1.05 E-19**	0.34
		4-allylphenol sulfate	Xenobiotics	Food Component/Plant	**1.07 E-11**	0.27
F	**1.39 E-10**	4-guanidinobutanoate	Amino Acid	Guanidino and Acetamido Metabolism	**5.80 E-20**	0.34
		Formiminoglutamate	Amino Acid	Histidine Metabolism	**6.11 E-40**	0.46
		Hydantoin-5-propionic acid	Amino Acid	Histidine Metabolism	**3.72 E-60**	0.67
		2-oxoarginine	Amino Acid	Urea cycle; Arginine and Proline Metabolism	**1.85 E-16**	0.25
		Argininate	Amino Acid	Urea cycle; Arginine and Proline Metabolism	**1.38 E-40**	0.38
G	**1.34 E-07**	Hexadecenedioate (C16:1-DC)	Lipid	Fatty Acid, Dicarboxylate	**9.90 E-32**	0.41
		Hexadecanedioate (C16)	Lipid	Fatty Acid, Dicarboxylate	**1.01 E-21**	0.34
		Octadecadienedioate (C18:2-DC)	Lipid	Fatty Acid, Dicarboxylate	**1.75 E-23**	0.32
H	**1.15 E-05**	Isovalerylglycine	Amino Acid	BCAA Metabolism	**6.70 E-40**	0.48
		Isobutyrylglycine (C4)	Amino Acid	BCAA Metabolism	**4.61 E-35**	0.41
		Isobutyrylcarnitine (C4)	Amino Acid	BCAA Metabolism	**5.57 E-23**	0.37

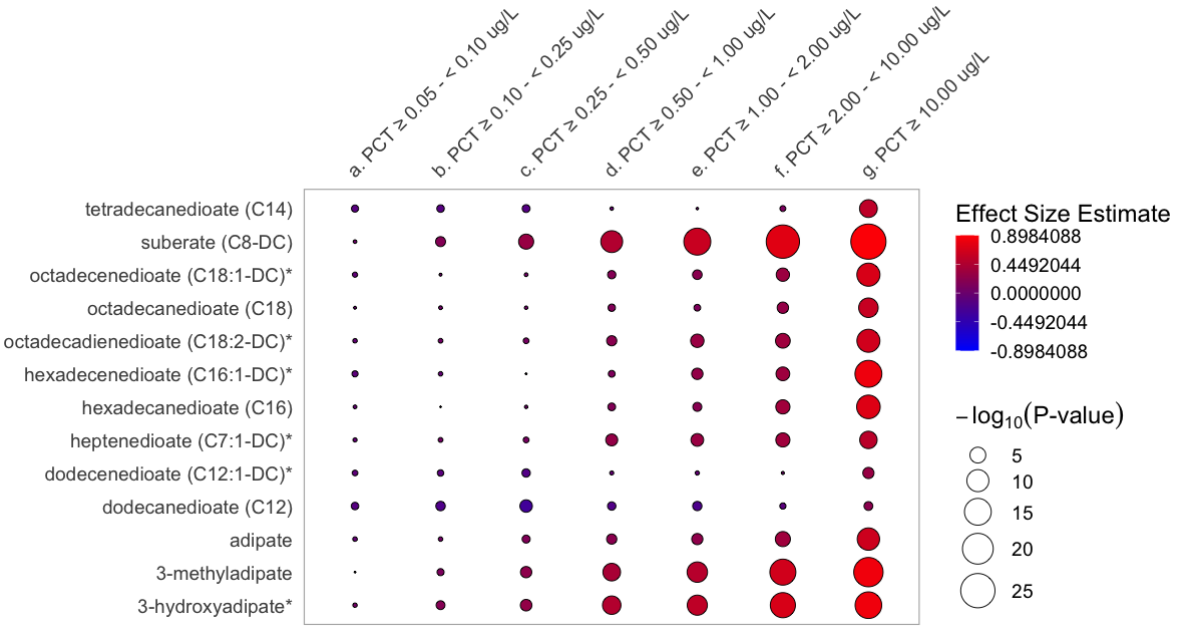
Note: Module P-value is the Bonferroni adjusted P-value of the GGM module; Metabolite is the Name of the metabolite in module; Super Pathway is the Name of the major biochemical pathway in the module; Sub-pathway is a subset of a the major biochemical pathway in the module; Component P-value and β coefficient results presented following individual mixed effects modeling of each of the 659 individual metabolites measured at day 0, 3 and 7 with a binary exposure of

PCT < 0.5 ug/L or ≥ 0.5 ug/L. All estimates adjusted for age, SAPS II, admission diagnosis, 25(OH)D at randomization, absolute change in 25(OH)D level at day 3 and plasma day (as the random-intercept). A multiple test-corrected threshold of $P < 7.59 \times 10^{-5}$ was used to identify all significant associations shown in bold.

Supplemental Figure 1. Consort Diagram: 1212 plasma samples from 444 subjects in VITdAL-ICU randomized clinical trial

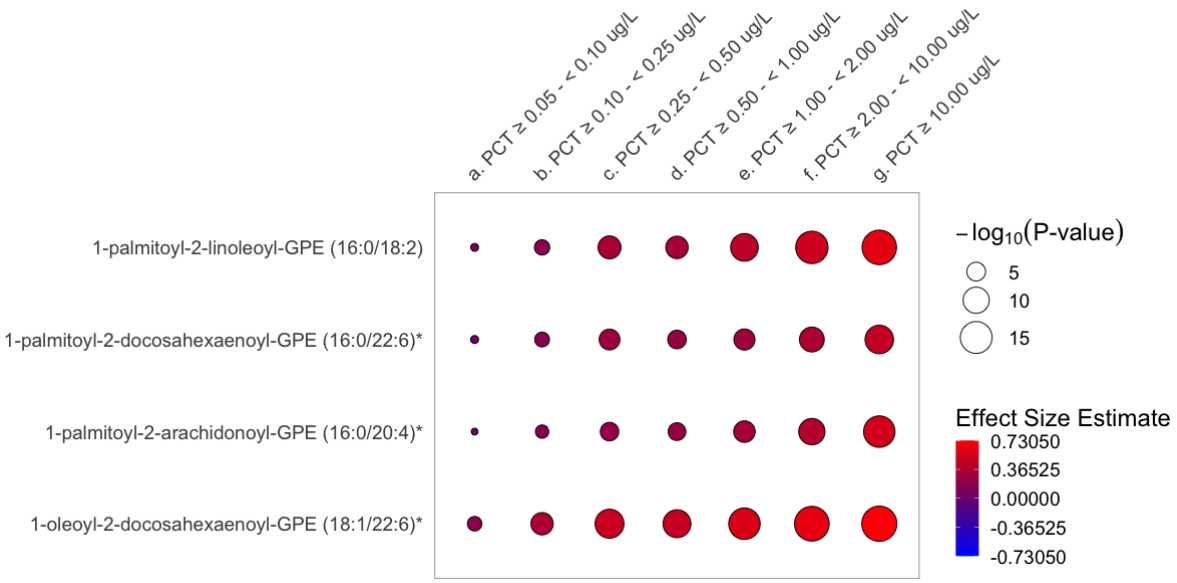


Supplemental Figure 2. Rain Plot of repeated measures dicarboxylate fatty acids metabolomics data (day 0, 3 and 7) relative to PCT level



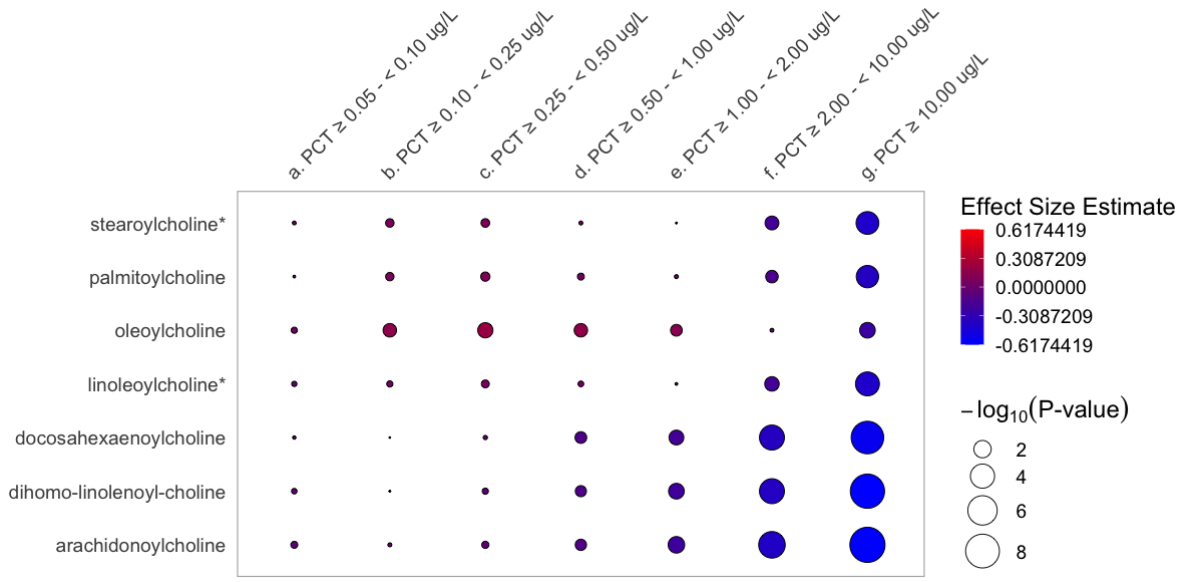
Correlations between individual dicarboxylate fatty acid metabolites and PCT level groups at day 0, 3 or 7 were determined utilizing linear regression models correcting for age, SAPS II, admission diagnosis, 25(OH)D at day 0 and for absolute change in 25(OH)D level at day 3. The magnitude of beta coefficient estimates is shown by a color fill scale and the corresponding significance level ($-\log_{10}$(P-value)) is represented by size of the circle. The intensity of the red fill color represents an increase in effect size for that metabolite in PCT level groups compared to subjects with PCT <0.05 ug/L. The intensity of the blue fill color represents a decrease in effect size for that metabolite in PCT level groups compared to subjects with PCT <0.05 ug/L. Statistical significance is the multiple test-corrected threshold of $-\log_{10}$(P-value) > 1.45 which is equivalent to FDR < 0.05.

Supplemental Figure 3. Rain Plot of repeated measures phosphatidylethanolamine metabolomics data (day 0, 3 and 7) relative to PCT level



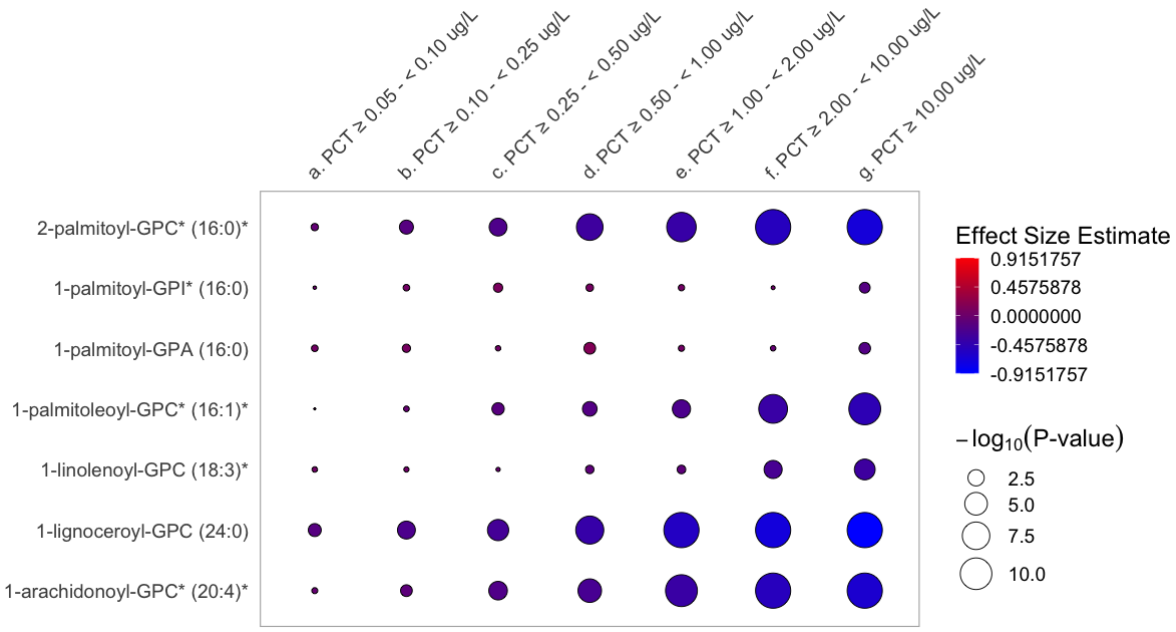
Correlations between individual phosphatidylethanolamine metabolites and PCT level groups at day 0, 3 or 7 were determined utilizing linear regression models correcting for age, SAPS II, admission diagnosis, 25(OH)D at day 0 and for absolute change in 25(OH)D level at day 3. The magnitude of beta coefficient estimates is shown by a color fill scale and the corresponding significance level ($-\log_{10}$(P-value)) is represented by size of the circle. The intensity of the red fill color represents an increase in effect size for that metabolite in PCT level groups compared to subjects with PCT <0.05 ug/L. The intensity of the blue fill color represents a decrease in effect size for that metabolite in PCT level groups compared to subjects with PCT <0.05 ug/L. Statistical significance is the multiple test-corrected threshold of $-\log_{10}$(P-value) > 1.45 which is equivalent to FDR < 0.05.

Supplemental Figure 4. Rain Plot of repeated measures acylcholine metabolomics data (day 0, 3 and 7) relative to PCT level



Correlations between individual acylcholine metabolites and PCT level groups at day 0, 3 or 7 were determined utilizing linear regression models correcting for age, SAPS II, admission diagnosis, 25(OH)D at day 0 and for absolute change in 25(OH)D level at day 3. The magnitude of beta coefficient estimates is shown by a color fill scale and the corresponding significance level ($-\log_{10}$(P-value)) is represented by size of the circle. The intensity of the red fill color represents an increase in effect size for that metabolite in PCT level groups compared to subjects with PCT <0.05 ug/L. The intensity of the blue fill color represents a decrease in effect size for that metabolite in PCT level groups compared to subjects with PCT <0.05 ug/L. Statistical significance is the multiple test-corrected threshold of $-\log_{10}$(P-value) > 1.45 which is equivalent to FDR < 0.05. Supplemental Figure 4. Rain Plot of repeated measures lysophospholipid metabolomics data (day 0, 3 and 7) relative to PCT level.

Supplemental Figure 5. Rain Plot of repeated measures lysophospholipid metabolomics data (day 0, 3 and 7) relative to PCT level

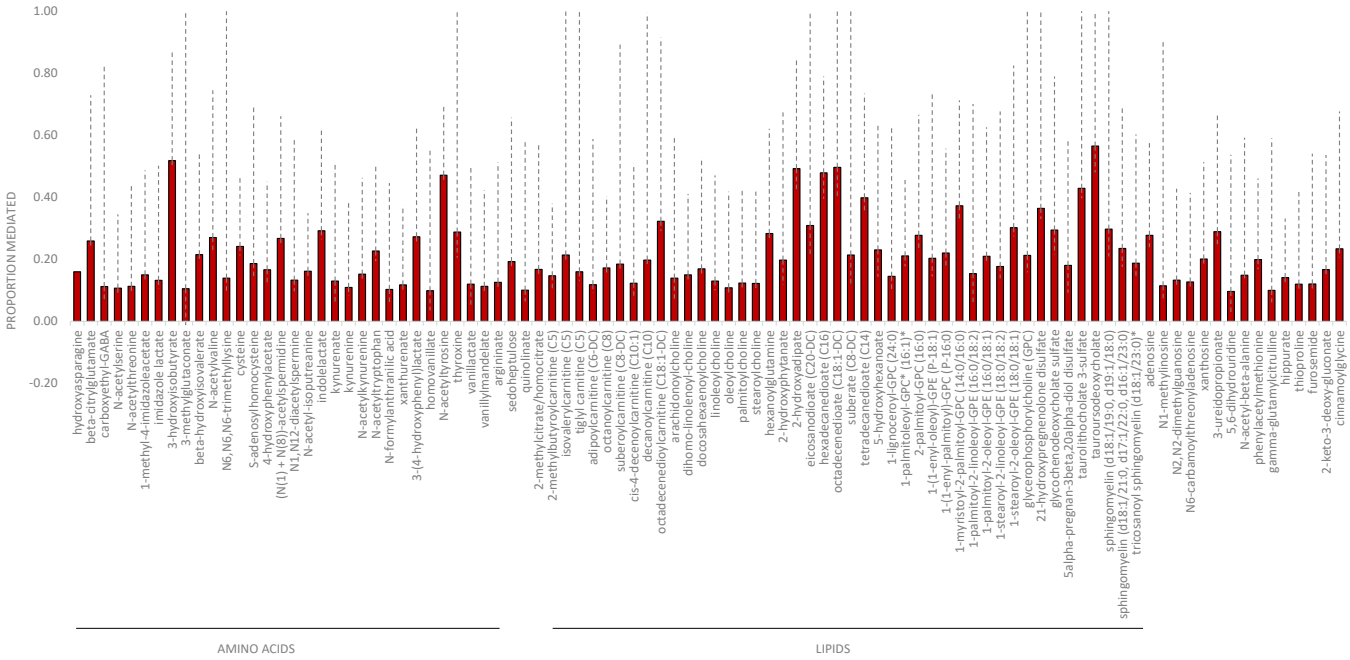


Correlations between individual lysophospholipid metabolites and PCT level groups at day 0, 3 or 7 were determined utilizing linear regression models correcting for age, SAPS II, admission diagnosis, 25(OH)D at day 0 and for absolute change in 25(OH)D level at day 3. The magnitude of beta coefficient estimates is shown by a color fill scale and the corresponding significance level ($-\log_{10}$(P-value)) is represented by size of the circle. The intensity of the red fill color represents an increase in effect size for that metabolite in PCT level groups compared to subjects with PCT <0.05 ug/L. The intensity of the blue fill color represents a decrease in effect size for that metabolite in PCT level groups compared to subjects with PCT <0.05 ug/L. Statistical significance is the multiple test-corrected threshold of $-\log_{10}$(P-value) > 1.45 which is equivalent to FDR < 0.05.

Supplemental Figure 6. Relative abundance of plasma metabolites in branched chain amino acids

Note: Boxplots show relative abundance of plasma BCAAs (A-C), BCKAs (D-F), pre BCKDH metabolites of BCKAs (G-I), metabolites downstream from BCKDH (J-T) and BCAA-derived carnitines (J, K, O, S). Metabolite relative plasma abundance is shown at day 0, 3 and 7 for subjects with PCT<0.5 ug/L or ≥0.50 ug/L. Unadjusted metabolite abundance data was normalized in terms of raw area counts and underwent a cube root transformation followed by Pareto scaling. U. Catabolic pathways of BCAAs and involved enzymes (in grey boxes). Metabolites highlighted by circled letters are shown in figures A-Q. Grey circled letters are the metabolite indicated, white circled letters are metabolites of the indicated metabolite (i.e. 4-methyl-2-oxopentanoate is shown in D and alpha-hydroxyisovalerate a metabolite of 4-methyl-2-oxopentanoate is shown in G). Abbreviations: 3MGH, 3-Methylglutaconyl-Coenzyme-A Hydratase; ACADSB, short/branched chain acyl-CoA dehydrogenase; BCAT, branched chain amino transferase; BCKDH, branched chain amino acid dehydrogenase; HIBDH, 3-hydroxyisobutyrate dehydrogenase; HIBCH, 3-hydroxyisobutyryl-CoA hydrolase; HMG-CoA lyase, 3-hydroxy-3-methylglutaryl-CoA lyase; IBD, Isobutyryl-CoA dehydrogenase; IVD, isovaleryl-CoA dehydrogenase; MCC, methylcrotonoyl-CoA carboxylase; MMSDH, Mammalian Methylmalonate-Semialdehyde Dehydrogenase; SCHMAD: short chain hydroxymethylacyl-CoA dehydrogenase.

Supplemental Figure 7. Proportion mediated by total serum bilirubin for metabolites



Proportion mediated (bars) with 95% confidence intervals by total serum bilirubin for individual metabolites significantly associated with Procalcitonin in linear regression analyses.

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Manuscript 2

Creatinine Metabolomics in the Critically Ill: a post-hoc metabolomics cohort study of the VITdAL-ICU trial

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ABSTRACT

Background and objectives: Creatinine is a general biomarker of kidney function and small changes of creatinine imply acute kidney dysfunction in acute critical illness. The metabolomic profile regarding acute increasing creatinine in critical illness is unclear. We aimed to investigate whether if the metabolomic profile of critical illness differs with an elevation of creatinine levels in adults.

Design, setting, participants, and measurements: This was a post-hoc metabolomics study of the Correction of Vitamin D Deficiency in Critically Ill Patients (VITdAL-ICU) randomized controlled trial at a university hospital in Austria. The abundance of 659 metabolites in 1213 plasma samples from 444 patients at randomization (day 0), day 3, and day 7 were analyzed. Exposure of interest was serum creatinine levels at each point. Student's t-test, orthogonal partial least square-discriminant analysis, linear mixed-effects models with Bonferroni multiple testing correction were performed regarding creatinine levels.

Results: The median [IQR] serum creatinine at day 0 was 1.1 [0.8, 1.8] mg/dL. In linear mixed-effects models in longitudinal data, 334 individual metabolites were significantly associated with increased serum creatinine. Multiple metabolites of the acylcarnitine, dicarboxylate fatty acid, branched-chain amino acid, and phosphatidylethanolamine pathways had significant positive associations with elevated creatinine. Further, multiple members of acylcholine, lysophospholipid metabolite classes were significantly decreased with elevated creatinine. Of significant metabolites, 165 metabolites had not been reported as uremic solutes.

Conclusions: Acute metabolomic responses in critical illness were different in patients with elevated creatinine levels. Such metabolic profile alterations with increases in creatinine may represent mitochondrial stress and impaired energy utilization in mitochondria.

Keywords

Metabolomics, Creatinine, Critical illness, Acylcarnitine, Dicarboxylate fatty acids, Branched chain amino acids, Pentose

List of non-standard abbreviations

VITdAL-ICU trial - Correction of Vitamin D Deficiency in Critically Ill Patients trial; OPLS-DA - orthogonal partial least square-discriminant analysis

INTRODUCTION

Acute kidney injury (AKI) is a major complication and a risk of death in patients with critical illness in an intensive care unit (ICU). AKI based on rises in serum creatinine from baseline value and decreases in urine output are widely used to identify and evaluate acute kidney dysfunction (1). Over 50% of the critically ill patients experienced AKI during the first week of their ICU stay (2). The common causes of AKI are sepsis, cardiac disease and cardiac surgery, and hepatorenal syndrome (3). Acute kidney dysfunction itself and its severity defined by creatinine is independently associated with increases in mortality (4).

Mitochondrial injury and the alteration of metabolism in mitochondria are observed in AKI. Research has demonstrated swollen mitochondria and mitochondrial fragmentation appear in the development of experimental AKI (5,6). AKI is shown to cause mitochondrial homeostasis changes such as the expression of mitochondrial respiratory proteins in rodents (6,7). Uremic solutes, which accumulate with kidney failure, also induce upregulation of glycolysis as an antioxidative stress response, causing mitochondrial TCA cycle downregulation and ATP shortage in mice (8). As mitochondria are the main energy source of the cell, such mitochondrial changes in structure and metabolism during AKI or chronic kidney disease (CKD) may induce impaired energy utilization.

The study of metabolomics exhaustively analyzes small molecules in biospecimens and is utilized to illustrate metabolic homeostasis including energy utilization. Previous metabolomic investigations on blood in patients after cardiac surgery revealed that carnitine and amino acids were associated with AKI (9,10).

Though identification of AKI in surgical patients can be robust due to known baseline serum creatinine value, one of the crucial problems regarding the identification of AKI is missing information on stable and baseline creatinine value in general critically ill patients before admission. Therefore, metabolomic profiling on blood metabolites associated with acute kidney dysfunction in nonspecific critically ill patients remains uncertain.

Serum creatinine is a major biomarker of kidney function for estimation of glomerular filtration rate (GFR) and used worldwide to assess kidney function in research and clinical practice. A small increase of serum creatinine is one criterion of AKI from baseline and a decrease in GFR is similarly one criterion of chronic kidney disease (CKD) (11). In patients with CKD, metabolomic profiling is notable for associations between the longitudinal decrease of estimated glomerular filtration rate (eGFR) and the abundance of acylcarnitines and phosphatidylcholines (12,13). However, among patients in acute phases like critical illness, metabolomic profiling regarding the alteration of serum creatinine is unclear.

Previous metabolomic studies suggested disturbance of metabolic homeostasis might be representative of the presence and severity of kidney dysfunction and energy utilization. We hypothesize that a specific plasma metabolomic profile is different according to the acute changes of serum creatinine levels in patients with critical illness. As a post-hoc metabolomics study, we analyzed 659 metabolites in 1213 plasma samples from 444 subjects collected during the VITdAL-ICU trial (14). We assessed the associations between increased creatinine and individual metabolites and plasma metabolite families among three-time points early in the course of critical illness.

Additionally, we compared significantly altered individual metabolites with uremic solutes reported in the previously published (15).

MATERIALS AND METHODS

Detailed trial and metabolomics methods are reported in Supplemental Methods. In short, the VITdAL-ICU trial (NCT01130181) randomly assigned 475 adult patients with critical illness to vitamin D_3 or placebo once at a dose of 540,000 IU followed by 90,000 IU monthly (14). Hospital length of stay was the primary endpoint. Whole blood was collected at randomization (day 0), day 3, and day 7. Written informed consent was gained at the time of enrollment. The Partners Human Research Committee at the Brigham and Women's Hospital approved the protocol of the post-hoc metabolomics study (Protocol # 2015P002766).

Serum creatinine measured at randomization was the exposure of interest. Frozen plasma from 453 trial participants was available for analysis. Nine participants who did not have serum creatinine measured at randomization were excluded. A total of 1213 plasma samples from 444, 402, and 367 patients at day 0, day 3, and day 7 were analyzed by four ultra-high-performance liquid chromatography/ tandem accurate mass spectrometry methods (Metabolon, Inc. in 2017), respectively (**Figure 1**) (16). Metabolomic profiling identified 983 plasma metabolites. We removed metabolites with the lowest interquartile range of variability for reduction of baseline noise, leaving 659 metabolites to analyze. Each metabolite raw area count data was normalized, cube root transformed, and Pareto scaled to produce abundance data that were on the same scale with an approximately normal distribution.

For univariable analysis of day 0 data, Student's t-test was performed to discover the significant change of individual metabolite between two groups divided as the median serum creatinine threshold with a Bonferroni multiple testing correction

threshold of P-value <7.59 × 10^{-5} (0.05/659 metabolites per plasma sample) through MetaboAnalyst (17). Orthogonal partial least square-discriminant analysis (OPLS-DA) was conducted to evaluate the significance of classification discrimination between two groups on day 0 metabolomic profile. In addition, we validated the OPLS-DA model with permutation testing.

For longitudinally measured data, we used linear mixed-effects models to find the association between the relative abundance of individual metabolites (as an outcome) and serum creatinine levels (as a continuous exposure) at day 0, 3, and 7 adjusting for age, sex, baseline 25(OH)D, an absolute increase in 25(OH)D at day 3, SAPS II, admission diagnosis, plasma day to control the effects of time, and patient identification as individual patient-specific random-intercept (Model 1). We excluded 25 patients without serum 25(OH)D level measured at day 3 which is utilized to mitigate the effect of the trial intervention (18−20). We analyzed a total of 1188 plasma samples from 419 patients at day 0, 402 patients at day 3, and 367 patients at day 7 with a Bonferroni multiple testing correction threshold of P-value <7.59 × 10-5. All mixed-effects linear regression analyses were performed by STATA/IC 15.1 (College Station, TX).

For sensitivity analysis, we repeated the multivariable linear mixed-effects model (Model 1) with exclusion of End Stage Renal Disease patients on hemodialysis at baseline. Further, as inflammation is a major cause of AKI, we additionally adjusted Model 1 for serum procalcitonin levels. Again, Bonferroni corrected P-values <7.59 × 10^{-5} were considered significant.

RESULTS

In the cohort (N=444), the median [IQR] of creatinine at day 0 was 1.1 [0.8, 1.8] mg/dL, 11% had AKI by day 7 and 5% had pre-existing end-stage renal disease (ESRD) defined as kidney disease requiring renal replacement therapy. Baseline characteristics such as age, sex, SAPS II score, ICU type, admission diagnosis, 25(OH)D level at day 0, and hemodialysis at baseline were significantly different between subjects grouped by creatinine threshold (**Table 1, Supplemental Table 1**). Differences in intervention status, the absolute change in 25(OH)D level at day 3 and AKI by day 7 were not significant. The 28-day mortality of the lower creatinine group and higher creatinine group were significantly different (17% and 35%, respectively).

In plasma samples at day 0 (N=444), 313 individual metabolites showed significant crude differences between subjects with or without creatinine ≥1.1 mg/dL (all Bonferroni corrected P-value $<7.59 \times 10^{-5}$). The differences existed in increases of multiple metabolite members of the acylcarnitine, amino acids including branched-chain amino acid, dicarboxylate fatty acid, phosphatidylethanolamine pathways, and decreases in metabolites from the lysophospholipid pathways. Metabolomic profiles between subjects with and without creatinine ≥1.1 mg/dL at day 0 demonstrated acceptable predictability (Q2 value 0.482) regarding the OPLS-DA model (**Supplemental Table 2**).

Regarding the longitudinal 1188 plasma samples collected at day 0, 3, and 7 from 419 VITdAL-ICU trial subjects, mixed-effects modeling revealed 334 individual metabolites were significantly associated with creatinine. Three hundred metabolites had positive associations with creatinine, including acylcarnitines, branched-chain

amino acids, phosphatidylethanolamines, as well as the pentose phosphate and dicarboxylate fatty acid pathways (**Summarized in Table 2, full data in Supplemental Table 3**). Thirty-four metabolites were negatively associated with creatinine, dominated by multiple members of acyl choline and lysophospholipid pathway metabolites (**Summarized in Table 3, full data in Supplemental Table 4**). Of the significant 334 metabolites, 169 metabolites were previously reported as uremic solutes in ESRD patients, and 165 metabolites were not previously reported as known uremic solutes (15) (**Supplemental Table 3 & 4**). Metabolites associated with elevated creatinine not previously reported include those in the phosphatidylethanolamine, lysophospholipid, acyl choline, as well as primary- and secondary-bile acid metabolism pathways (**Table 4**). Addition of procalcitonin to the original linear mixed effects model did not materially alter the significance or direction of associations between creatinine and individual metabolites (**Supplemental Table 5**).

In sensitivity analysis, the linear mixed effects modeling in the longitudinal 1129 plasma samples from the cohort excluding ESRD patients showed that 338 individual metabolites were significantly associated with creatinine (**Supplemental Table 3 & 4**). The vast majority of the significant metabolites present following exclusion of ESRD patients were also present in the cohort including ESRD patients (325 of 338). Thirteen additional metabolites were noted when ESRD patients were excluded (**Supplemental Table 6)**.

DISCUSSION

Our metabolomics study revealed groups of metabolites along similar sub-pathways are strongly associated with increasing creatinine levels. Multiple representatives of phosphatidylethanolamines, branched-chain amino acids (BCAA), acylcarnitines, pentose phosphate and dicarboxylate pathway metabolites, had positive associations with elevated creatinine levels. Further, we show that multiple representatives of lysophospholipid and acylcholine metabolites had a significant negative association with creatinine, including multiple representatives of lysophospholipid and acylcholine. We highlighted the alterations of the metabolites with increasing creatinine levels that were not previously identified in ESRD.

Evidence supports the hypothesis that increases in uremic metabolites have the potential to impair mitochondrial function (21−25). When compared to healthy adults, patients with CKD are shown to have mitochondrial dysfunction with downregulation of peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α) an important regulator of energy metabolism and mitochondrial biogenesis (25−32). Importantly, both animal models and patients with CKD are shown to have decreased activity of mitochondrial citrate synthase and hydroxyacyl-CoA dehydrogenase in addition to prolonged skeletal muscle phosphocreatine recovery time following exertion (32).

A large proportion of our observed significant metabolites indicate alterations of energy utilization, especially in mitochondria. Plasma short-chain acylcarnitines (C2-C7) were consistently increased in our mixed-effects results. Short-chain acylcarnitines are elevated in plasma when mitochondrial fatty acid β-oxidation is incomplete and are indicative of mitochondrial dysfunction (33). Acylcarnitine metabolites are frequently

reported as associated with kidney dysfunction (10,12,34). We also find that dicarboxylate fatty acids are prominent in plasma with increases in creatinine. Dicarboxylate fatty acids are generated when mitochondrial fatty acid β-oxidation is impaired via fatty acid ω-oxidation that occurs in the smooth endoplasmic reticulum of the liver and kidney (35). Additionally, we note pentose phosphate pathway metabolites are significantly increased with elevations in creatinine. Evidence suggests that the pentose phosphate pathway acts as a “metabolic redox sensor” upregulated during oxidative stress and is suggestive of a metabolic shift away from mitochondrial beta oxidation (36−39).

In amino acid pathways, the metabolism of the branched-chain amino acids (BCAAs) included the largest number of the altered metabolites in the amino acid class. BCAAs are transformed to acetyl-CoA or succinyl-CoA as energy resources when mitochondrial fatty acid β-oxidation is deficient (40). Additionally, with increased creatinine we observe elevations of the short-chain acylcarnitines C3, C4 and C5 which are generated during catabolic metabolism of BCAAs (40,41). The number of mitochondria in the kidney is second only to the heart (42). Impaired mitochondrial function has an important role as an initiator of and contributor to acute kidney injury (43). Therefore, our findings of increased circulating acylcarnitines, dicarboxylate fatty acids, BCAA catabolic and pentose phosphate pathway metabolites with increasing creatinine indicate a change of energy utilization, especially in mitochondria (**Figure 2**).

Our observations show decreases of acylcholine and lysophospholipid metabolite classes were associated with increasing creatinine. Though poorly understood, evidence supports that acylcholines are fatty acid analogs of acetylcholine

that serve as endogenous modulators of the acetylcholine system (44). Acetylcholine regulates immune-mediated inflammation (45,46). Lysophospholipids such as lysophosphatidylcholine are proinflammatory lipids that activate monocytes, macrophages, and T cells through specific cell-surface lysophospholipid receptors (47). Inflammation is one of the important etiologies of acute kidney injury. The lower levels of metabolites of acylcholine and lysophospholipid classes associated with increased creatinine in our study may reflect renal dysfunction due to systemic inflammation

Our findings of associations between serum creatinine levels and individual metabolites were robust regardless of serum procalcitonin levels. Procalcitonin is a common biomarker of the severity of systemic inflammation and sepsis (48). Sepsis and inflammation due to other medical conditions are the main reasons for AKI (3). Therefore, the changes of metabolites might be representative severity of inflammation rather than AKI. However, we revealed the metabolite alterations were strongly and significantly associated with serum creatinine levels despite adjusting serum procalcitonin levels in addition to the multivariable linear mixed effects model.

Our research has multiple strengths in methodology. For longitudinal metabolomics data with several clinical variables at repeated time points, linear mixed-effects models are robust statistical analyses considering autocorrelation in each patient (49). We could focus on metabolites' alterations relative to creatinine in spite of simple changes under the course of critical illness or trial procedure due to this technique (19). To reduce false positive observations, we applied Bonferroni correction that is conservative significance threshold for multiple comparisons in our adjusted mixed-effects model. Additionally, we compared individual metabolites associated with the

change of creatinine to a large number of reported uremic solutes and found unreported metabolites in the context of elevated creatinine.

There are several potential limitations in our study. Creatinine does not rapidly reflect a decrease or recovery in renal function, but also is affected by muscle mass. Although other biomarkers like cystatin C are less likely to be affected by time lag and lean tissue mass, we could not measure other biomarkers to detect acute renal dysfunction as well as muscle mass (50, 51). Thus, our findings in alterations of each metabolite may be a later response for change of renal function and be affected by other factors such as muscle mass. Additionally, we could not obtain the baseline serum creatinine value before ICU admission to evaluate the alterations of renal function from the normal state prior to critical illness. Therefore, patients might have acute renal dysfunction at randomization. Besides, the incidence of AKI by day 7 was similar between the subject's groups with high or low serum creatinine due to unreliable diagnosis of AKI at ICU admission. However, we demonstrated significant linear associations between acute incremental changes of serum creatinine and individual metabolites by applying linear mixed-effects models to longitudinal data over seven days. Moreover, we could not obtain the timing of renal replacement therapy and blood test in a day. Serum creatinine value decreases after or during renal replacement therapy and individual metabolites can be affected by renal replacement therapy. Furthermore, as observational study, our findings should be regarded as hypothesis generating rather than causality. We could not exclude the effect of unknown confounders despite multivariable adjustment because of our post-hoc analysis of nonrandomized comparisons. In addition, our original trial recruited white critically ill

patients from a single large academic medical center. Creatinine and estimate glomerular filtration rate based on serum creatinine may be affected by race besides age, sex, and muscle mass (52). This may mean limited generalizability to all critically ill patients. Lastly, though the biological function and association of highlighted metabolites have been explored, it is still unclear how a change in metabolite abundance significantly affects clinical status.

In summary, metabolomic profiling of critically ill patients demonstrated 331 metabolites were strongly associated with elevating serum creatinine. Metabolomic alterations with increasing creatinine indicate mitochondrial dysfunction and impaired energy utilization such as fatty acid β-oxidation. Our findings provide a basis for future research into the pathophysiology and the dynamics of AKI and energy utilization in mitochondria.

FIGURES

Figure 1. Consort Diagram: 1213 plasma samples from 444 subjects in VITdAL-ICU randomized clinical trial

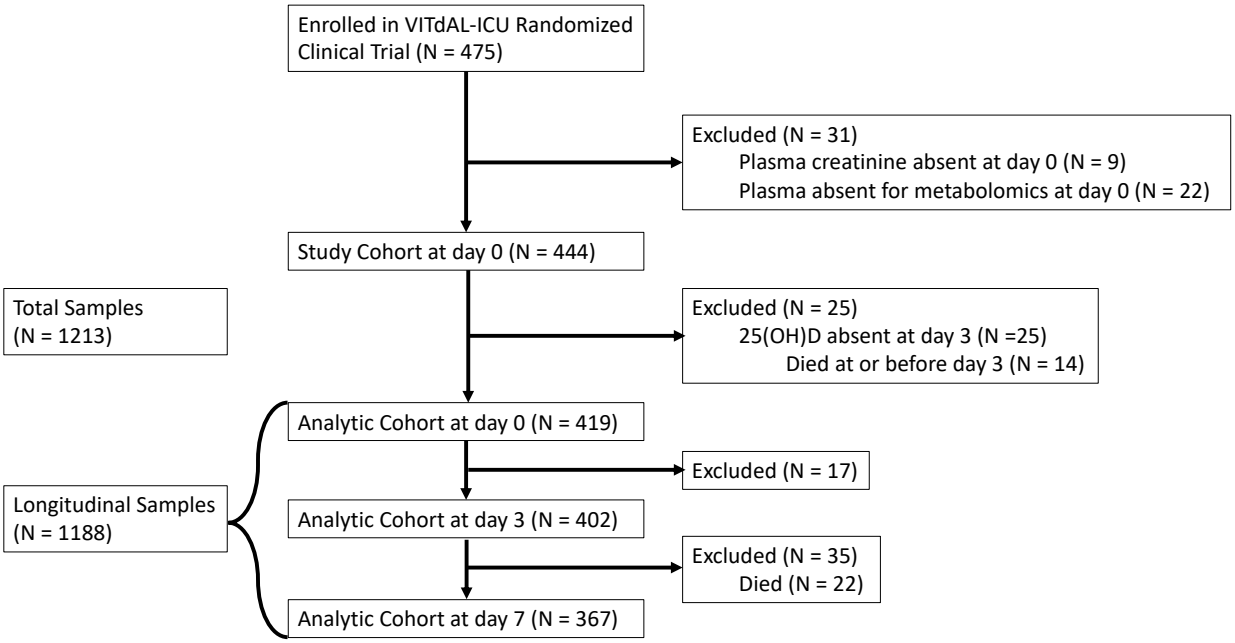
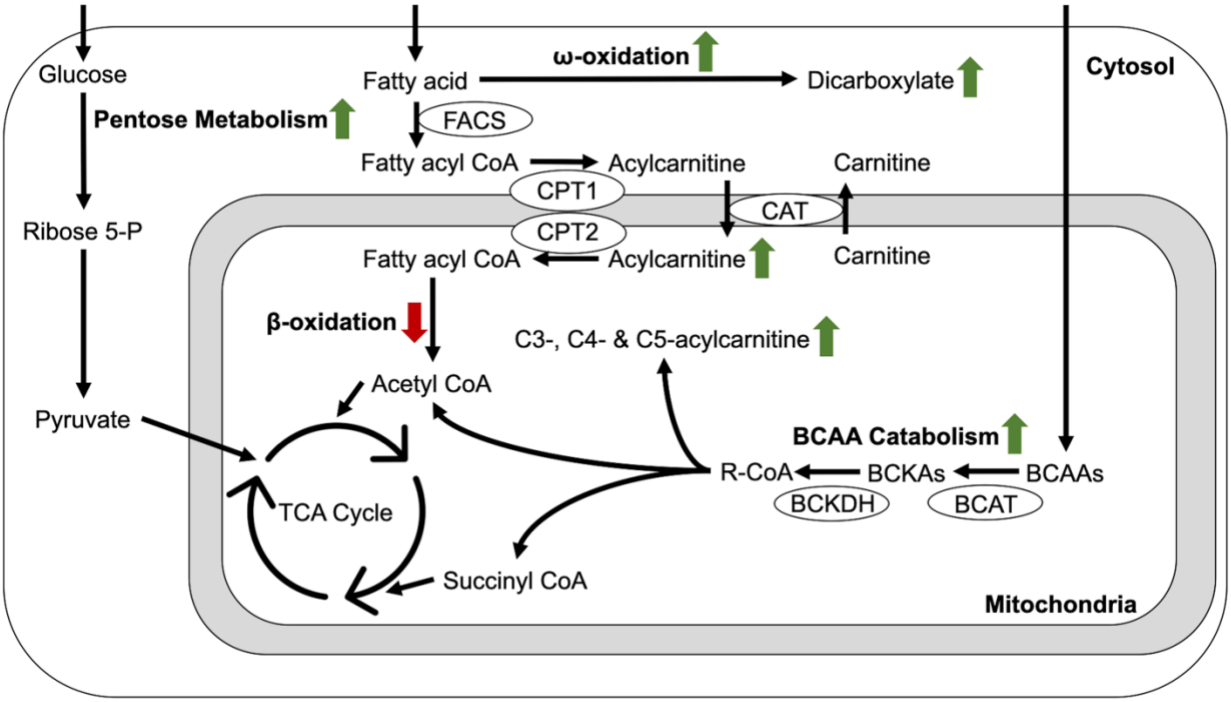


Figure 2. Energy metabolism changes regarding increases of serum creatinine



BCAAs, Branched chain amino acids; BCKAs, Branched chain alpha-keto acids; R-CoA, Acyl-coenzyme; BCAT, Branched chain aminotransferase; BCKDH, Branched chain alpha-keto acid dehydrogenase complex; FACS, Fatty acyl-CoA synthase; CPT1, Carnitine palmitoyl transferase 1; CPT2, Carnitine palmitoyl transferase 2; CACT, Carnitine-acylcarnitine translocase; TCA, Tricarboxylic acid.

TABLES

Table 1. Baseline Characteristics on Day 0 by Creatinine levels

Characteristic	Overall n = 444	Cr < 1.1 mg/dL n = 221	Cr ≥ 1.1 mg/dL n = 223	P-value
Age, yr (mean (SD))	64.8 (14.7)	61.0 (16.1)	68.6 (12.0)	< 0.001
Female, n (%)	155 (35)	94 (43)	61 (27)	0.001
Charlson Comorbidity Index (median [IQR])	3.0 [1.0, 4.3]	2.0 [1.0, 3.0]	4.0 [2.0, 5.0]	<0.001
Hemodialysis at baseline, n (%)	22 (5)	3 (1)	19 (9)	0.001
SAPS ll (mean (SD))	33.5 (15.4)	31.8 (16.0)	35.2 (14.7)	0.023
ICU type, n (%)				< 0.001
Anesthesia, n (%)	83 (19)	43 (20)	40 (18)	
Cardiac Surgery, n (%)	130 (29)	35 (16)	95 (43)	
Medical, n (%)	99 (22)	48 (22)	51 (23)	
Neurological, n (%)	109 (25)	83 (38)	26 (12)	
Surgical, n (%)	23 (5)	12 (5)	11 (5)	
25(OH)D, ng/mL (mean (SD))	13.9 (8.8)	14.0 (5.5)	13.7 (11.1)	0.691
Vitamin D3 intervention, n (%)	219 (49)	108 (49)	111 (50)	0.923
Change in 25(OH)D Day 0 to Day 3, ng/mL (median [IQR])	3.1 [0.0, 16.5]	3.3 [-0.2, 20.5]	2.85 [0.0, 12.1]	0.365
Serum Creatinine, mg/dL (median [IQR])	1.1 [0.8, 1.8]	0.8 [0.6, 0.9]	1.8 [1.4, 2.3]	< 0.001
Serum procalcitonin, ng/mL (median [IQR])	0.7 [0.2, 2.7]	0.3 [0.1, 0.8]	1.7 [0.6, 6.1]	< 0.001
Acute kidney injury by day 7, n (%)	50 (11)	24 (11)	26 (12)	0.768
28-day mortality, n (%)	114 (26)	37 (17)	77 (35)	< 0.001

Table 2. Metabolites significantly increased with increased Creatinine over day 0-7

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log10p	Super Pathway	Sub Pathway
alpha-hydroxyisovalerate	0.084	2.00E-08	1.32E-05	7.70	Amino Acid	BCAA Metabolism
2-hydroxy-3-methylvalerate	0.090	7.25E-09	4.77E-06	8.14	Amino Acid	BCAA Metabolism
3-hydroxyisobutyrate	0.096	2.11E-10	1.39E-07	9.68	Amino Acid	BCAA Metabolism
beta-hydroxyisovalerate	0.144	9.71E-25	6.40E-22	24.01	Amino Acid	BCAA Metabolism
3-hydroxy-2-ethylpropionate	0.165	4.07E-35	2.68E-32	34.39	Amino Acid	BCAA Metabolism
N-acetylleucine	0.179	8.51E-38	5.61E-35	37.07	Amino Acid	BCAA Metabolism
methylsuccinate	0.180	3.39E-31	2.23E-28	30.47	Amino Acid	BCAA Metabolism
ethylmalonate	0.207	6.54E-49	4.31E-46	48.18	Amino Acid	BCAA Metabolism
N-acetylvaline	0.227	7.90E-102	5.21E-99	101.10	Amino Acid	BCAA Metabolism
2,3-dihydroxy-2-methylbutyrate	0.277	2.67E-70	1.76E-67	69.57	Amino Acid	BCAA Metabolism
isobutyrylglycine (C4)	0.286	3.10E-64	2.05E-61	63.51	Amino Acid	BCAA Metabolism
isovalerylglycine	0.292	1.07E-52	7.04E-50	51.97	Amino Acid	BCAA Metabolism
3-methylglutaconate	0.379	4.60E-216	3.03E-213	215.34	Amino Acid	BCAA Metabolism
alpha-hydroxyisovalerate	0.084	2.00E-08	1.32E-05	7.70	Amino Acid	BCAA Metabolism
propionylglycine (C3)	0.097	9.32E-09	6.14E-06	8.03	Lipid	BCAA Metabolism
methylmalonate (MMA)	0.217	8.40E-37	5.53E-34	36.08	Lipid	BCAA Metabolism
propionylcarnitine (C3)	0.156	2.22E-21	1.46E-18	20.65	Lipid	Acylcarnitine
butyrylcarnitine (C4)	0.095	6.81E-08	4.48E-05	7.17	Lipid	Acylcarnitine
isobutyrylcarnitine (C4)	0.255	1.26E-39	8.28E-37	38.90	Lipid	Acylcarnitine
2-methylbutyroylcarnitine (C5)	0.276	3.84E-64	2.53E-61	63.42	Lipid	Acylcarnitine
isovalerylcarnitine (C5)	0.187	5.56E-28	3.67E-25	27.25	Lipid	Acylcarnitine
tiglyl carnitine (C5)	0.293	3.18E-88	2.09E-85	87.50	Lipid	Acylcarnitine
3-methylglutarylcarnitine (C6-DC)	0.407	4.18E-170	2.75E-167	169.38	Lipid	Acylcarnitine
myristoylcarnitine (C14)	0.068	1.23E-05	8.09E-03	4.91	Lipid	Acylcarnitine
myristoleoylcarnitine (C14:1)*	0.070	3.76E-05	2.48E-02	4.43	Lipid	Acylcarnitine
3-hydroxyoleoylcarnitine (C18:1-OH)	0.080	1.87E-06	1.23E-03	5.73	Lipid	Acylcarnitine
laurylcarnitine (C12)	0.112	1.20E-12	7.92E-10	11.92	Lipid	Acylcarnitine

Table 2. Metabolites significantly increased with increased Creatinine over day 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway
octadecanedioylcarnitine (C18-DC)*	0.121	8.20E-13	5.40E-10	12.09	Lipid	Acylcarnitine
3-hydroxybutyrylcarnitine (1) (C4-OH)	0.134	4.37E-09	2.88E-06	8.36	Lipid	Acylcarnitine
5-dodecenoylcarnitine (C12:1)	0.137	2.17E-14	1.43E-11	13.66	Lipid	Acylcarnitine
acetylcarnitine (C2)	0.148	2.36E-26	1.55E-23	25.63	Lipid	Acylcarnitine
decanoylcarnitine (C10)	0.155	6.77E-23	4.46E-20	22.17	Lipid	Acylcarnitine
hexanoylcarnitine (C6)	0.170	1.64E-26	1.08E-23	25.78	Lipid	Acylcarnitine
octanoylcarnitine (C8)	0.178	9.66E-30	6.36E-27	29.02	Lipid	Acylcarnitine
octadecenedioylcarnitine (C18:1-DC)*	0.180	2.50E-19	1.65E-16	18.60	Lipid	Acylcarnitine
cis-4-decenoylcarnitine (C10:1)	0.188	3.66E-36	2.41E-33	35.44	Lipid	Acylcarnitine
3-hydroxybutyrylcarnitine (2) (C4-OH)	0.210	4.14E-38	2.73E-35	37.38	Lipid	Acylcarnitine
adipoylcarnitine (C6-DC)	0.333	2.00E-99	1.32E-96	98.70	Lipid	Acylcarnitine
suberoylcarnitine (C8-DC)	0.336	1.83E-78	1.20E-75	77.74	Lipid	Acylcarnitine
pimeloylcarnitine/3-methyladipoylcarnitine (C7-DC)	0.361	7.15E-143	4.71E-140	142.15	Lipid	Acylcarnitine
azelate (nonanedioate; C9)	0.087	4.42E-08	2.91E-05	7.35	Lipid	Fatty Acid, Dicarboxylate
maleate	0.103	6.08E-05	4.01E-02	4.22	Lipid	Fatty Acid, Dicarboxylate
adipate	0.133	2.63E-13	1.73E-10	12.58	Lipid	Fatty Acid, Dicarboxylate
3-carboxy-4-methyl-5-pentyl-2-furanpropionate (3-CMPFP)	0.153	1.14E-24	7.53E-22	23.94	Lipid	Fatty Acid, Dicarboxylate
suberate (C8-DC)	0.259	4.13E-64	2.72E-61	63.38	Lipid	Fatty Acid, Dicarboxylate
2-hydroxyadipate	0.263	1.44E-43	9.51E-41	42.84	Lipid	Fatty Acid, Dicarboxylate
heptenedioate (C7:1-DC)*	0.320	1.95E-82	1.28E-79	81.71	Lipid	Fatty Acid, Dicarboxylate
3-hydroxyadipate*	0.321	5.61E-73	3.70E-70	72.25	Lipid	Fatty Acid, Dicarboxylate
3-methyladipate	0.356	8.50E-123	5.60E-120	122.07	Lipid	Fatty Acid, Dicarboxylate
1,2-dilinoleoyl-GPE (18:2/18:2)*	0.083	3.70E-06	2.44E-03	5.43	Lipid	Phosphatidylethanolamine
1-palmitoyl-2-docosahexaenoyl-GPE (16:0/22:6)*	0.084	2.10E-11	1.39E-08	10.68	Lipid	Phosphatidylethanolamine

Table 2. Metabolites significantly increased with increased Creatinine over day 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	0.089	1.22E-10	8.07E-08	9.91	Lipid	Phosphatidylethanolamine
1-stearoyl-2-linoleoyl-GPE (18:0/18:2)*	0.089	3.40E-11	2.24E-08	10.47	Lipid	Phosphatidylethanolamine
1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)*	0.094	2.17E-13	1.43E-10	12.66	Lipid	Phosphatidylethanolamine
1-oleoyl-2-arachidonoyl-GPE (18:1/20:4)*	0.105	1.18E-15	7.80E-13	14.93	Lipid	Phosphatidylethanolamine
1-oleoyl-2-linoleoyl-GPE (18:1/18:2)*	0.105	1.28E-12	8.44E-10	11.89	Lipid	Phosphatidylethanolamine
1-stearoyl-2-oleoyl-GPE (18:0/18:1)	0.107	1.28E-11	8.40E-09	10.89	Lipid	Phosphatidylethanolamine
1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	0.110	1.00E-14	6.62E-12	14.00	Lipid	Phosphatidylethanolamine
1-oleoyl-2-docosahexaenoyl-GPE (18:1/22:6)*	0.111	3.83E-13	2.52E-10	12.42	Lipid	Phosphatidylethanolamine

Note: Using longitudinal data (day 0, 3, and 7), the association between relative quantitation of each individual metabolite noted above and Creatinine (Cr) levels over time were estimated utilizing linear mixed-effects models adjusting for age, sex, baseline 25(OH)D, an absolute increase in 25(OH)D, Simplified Acute Physiology Score (SAPS) II, plasma day, admission diagnosis and individual subjects (as the random-intercept). All significant mixed-effects associations have P-value $<7.59 \times 10^{-5}$. For the Acylcarnitines sub pathway: a capital C is followed by the number of carbons within the fatty acyl group attached to the carnitine. A colon followed by a number is one or more unsaturated carbons in the acylcarnitine ester (i.e. C10:1 is a monounsaturated C10 acylcarnitine). OH following the carbon number is a hydroxylic acylcarnitine. DC following the carbon number is a dicarboxylic acylcarnitine. GPE is glycerophosphoethanolamine.

Table 3. Metabolites significantly decreased with increased Creatinine over day 0-7

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway
arachidonoylcholine	-0.162	3.04E-18	2.00E-15	17.52	Lipid	Acyl Choline
dihomo-linolenoyl-choline	-0.156	3.07E-16	2.02E-13	15.51	Lipid	Acyl Choline
linoleoylcholine*	-0.154	4.89E-16	3.22E-13	15.31	Lipid	Acyl Choline
palmitoylcholine	-0.150	1.10E-15	7.25E-13	14.96	Lipid	Acyl Choline
stearoylcholine*	-0.150	1.27E-13	8.34E-11	12.90	Lipid	Acyl Choline
oleoylcholine	-0.143	1.01E-13	6.66E-11	13.00	Lipid	Acyl Choline
docosahexaenoylcholine	-0.142	1.86E-14	1.22E-11	13.73	Lipid	Acyl Choline
1-arachidonoyl-GPC* (20:4)*	-0.105	6.57E-16	4.33E-13	15.18	Lipid	Lysophospholipid
1-palmitoleoyl-GPC* (16:1)*	-0.083	2.53E-09	1.67E-06	8.60	Lipid	Lysophospholipid
1-linolenoyl-GPC (18:3)*	-0.081	1.69E-07	1.12E-04	6.77	Lipid	Lysophospholipid
2-palmitoyl-GPC* (16:0)*	-0.065	3.74E-06	2.47E-03	5.43	Lipid	Lysophospholipid
1-cerotoyl-GPC (26:0)*	-0.060	2.52E-05	1.66E-02	4.60	Lipid	Lysophospholipid

Note: Using longitudinal data (day 0, 3, and 7), the association between relative quantitation of each individual metabolite noted above and Creatinine (Cr) levels over time were estimated utilizing linear mixed-effects models adjusting for age, sex, baseline 25(OH)D, an absolute increase in 25(OH)D, Simplified Acute Physiology Score (SAPS) II, plasma day, admission diagnosis and individual subjects (as the random-intercept). All significant mixed-effects associations have P-value $<7.59 \times 10^{-5}$. GPC is glycerophosphorylcholine.

Table 4. Main pathway and the number of metabolites associated with increasing creatinine in mixed-effects models

Association	Sub pathway	The number of Metabolites as uremic solutes		
Positive	Acylcarnitine	24	New	13
			Known	11
	Branched Chain Amino Acids	16	New	10
			Known	6
	Phosphatidylethanolamines	10	New	10
			Known	0
	Primary bile acid metabolism	7	New	7
			Known	0
	Pentose metabolism	7	New	5
			Known	2
	Secondary bile acid metabolism	5	New	5
			Known	0
Negative	Acylcholine	7	New	7
			Known	0
	Lysophospholipid	5	New	5
			Known	0

SUPPLEMENTARY MATERIAL

Supplemental Tables

Supplemental Table 1. Additional Cohort Characteristics

Characteristic	Overall n = 444	Cr < 1.1 mg/dL n = 221	Cr ≥ 1.1 mg/dL n = 223	P-value
Admission Diagnosis				< 0.001
Brain Surgery No. (%)	4 (1)	4 (2)	0 (0)	
Cardiac surgery No. (%)	85 (19)	19 (9)	66 (30)	
Cardiovascular No. (%)	57 (13)	22 (10)	35 (16)	
Gastrointestinal/liver No. (%)	14 (3)	11 (5)	3 (1)	
Hematologic/oncologic No. (%)	2 (1)	1 (1)	1 (0)	
Metabolic No. (%)	3 (1)	2 (1)	1 (0)	
Neurologic No. (%)	107 (24)	80 (36)	27 (12)	
Other non-operative	3 (1)	2 (1)	1 (0)	
Other operative No. (%)	13 (3)	7 (3)	6 (3)	
Renal No. (%)	6 (1)	2 (1)	4 (2)	
Respiratory No. (%)	43 (10)	21 (10)	22 (10)	
Sepsis/infectious No. (%)	36 (8)	18 (8)	18 (8)	
Thoracic surgery No. (%)	13 (3)	3 (1)	10 (5)	
Transplantation No. (%)	12 (3)	3 (1)	9 (4)	
Trauma No. (%)	37 (8)	24 (11)	13 (6)	
Vascular surgery No. (%)	9 (2)	2 (1)	7 (3)	

Supplemental Table 2. At randomization (Day 0) OPLS-DA model goodness of fit and predictive ability

OPLS-DA		
R2X	R2Y	Q2
0.133	0.494	0.482

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
hydroxyasparagine	0.276	8.40E-200	5.54E-197	199.08	Amino Acid	Alanine and Aspartate Metabolism	x	x	x
creatine	0.075	6.29E-07	4.14E-04	6.20	Amino Acid	Creatine Metabolism		x	x
pyroglutamine*	0.085	2.53E-08	1.67E-05	7.60	Amino Acid	Glutamate Metabolism		x	x
4-hydroxyglutamate	0.088	2.78E-07	1.83E-04	6.56	Amino Acid	Glutamate Metabolism	x	x	x
citramalate	0.171	4.62E-29	3.05E-26	28.33	Amino Acid	Glutamate Metabolism	x	x	x
beta-citrylglutamate	0.188	2.48E-43	1.64E-40	42.61	Amino Acid	Glutamate Metabolism		x	x
N-acetylglutamate	0.214	7.21E-40	4.75E-37	39.14	Amino Acid	Glutamate Metabolism	x	x	x
carboxyethyl-GABA	0.221	8.36E-84	5.51E-81	83.08	Amino Acid	Glutamate Metabolism	x	x	x
alpha-ketoglutaramate*	0.261	5.17E-133	3.41E-130	132.29	Amino Acid	Glutamate Metabolism	x	x	x
N-acetyl-aspartyl-glutamate (NAAG)	0.274	1.99E-76	1.31E-73	75.70	Amino Acid	Glutamate Metabolism		x	x
N-acetylglutamine	0.279	2.40E-95	1.58E-92	94.62	Amino Acid	Glutamate Metabolism	x	x	x
cys-gly, oxidized	0.075	3.33E-05	2.19E-02	4.48	Amino Acid	Glutathione Metabolism		x	x
cysteinylglycine	0.249	5.00E-42	3.30E-39	41.30	Amino Acid	Glutathione Metabolism		x	x
N-acetylglycine	0.066	4.39E-05	2.90E-02	4.36	Amino Acid	Glycine, Serine and Threonine Metabolism			x
allo-threonine	0.116	8.69E-10	5.73E-07	9.06	Amino Acid	Glycine, Serine and Threonine Metabolism		x	x
N-acetylserine	0.297	6.43E-195	4.24E-192	194.19	Amino Acid	Glycine, Serine and Threonine Metabolism	x	x	x
N-acetylthreonine	0.310	3.51E-214	2.31E-211	213.45	Amino Acid	Glycine, Serine and Threonine Metabolism	x	x	x
4-guanidinobutanoate	0.144	6.98E-12	4.60E-09	11.16	Amino Acid	Guanidino and Acetamido Metabolism		x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
guanidinosuccinate	0.394	1.47E-111	9.71E-109	110.83	Amino Acid	Guanidino and Acetamido Metabolism	x	x	x
1-methylguanidine	0.519	6.33E-288	4.17E-285	287.20	Amino Acid	Guanidino and Acetamido Metabolism	x	x	x
N-acetylhistidine	0.158	1.21E-21	7.96E-19	20.92	Amino Acid	Histidine Metabolism	x	x	x
imidazole propionate	0.178	2.19E-11	1.44E-08	10.66	Amino Acid	Histidine Metabolism	x	x	x
formiminoglutamate	0.186	1.83E-23	1.21E-20	22.74	Amino Acid	Histidine Metabolism	x	x	x
imidazole lactate	0.213	4.99E-48	3.29E-45	47.30	Amino Acid	Histidine Metabolism	x	x	x
3-methylhistidine	0.226	5.85E-23	3.86E-20	22.23	Amino Acid	Histidine Metabolism	x	x	x
1-methyl-4-imidazoleacetate	0.287	2.78E-108	1.83E-105	107.56	Amino Acid	Histidine Metabolism	x	x	x
1-ribosyl-imidazoleacetate*	0.324	6.20E-147	4.09E-144	146.21	Amino Acid	Histidine Metabolism	x	x	x
N-acetylcarnosine	0.328	7.56E-125	4.99E-122	124.12	Amino Acid	Histidine Metabolism	x	x	x
hydantoin-5-propionic acid	0.332	3.93E-59	2.59E-56	58.41	Amino Acid	Histidine Metabolism	x	x	x
1-methylhistidine	0.342	3.82E-150	2.52E-147	149.42	Amino Acid	Histidine Metabolism	x	x	x
N-acetyl-1-methylhistidine*	0.440	2.70E-154	1.78E-151	153.57	Amino Acid	Histidine Metabolism	x	x	x
alpha-hydroxyisovalerate	0.084	2.00E-08	1.32E-05	7.70	Amino Acid	BCAA Metabolism		x	x
2-hydroxy-3-methylvalerate	0.090	7.25E-09	4.77E-06	8.14	Amino Acid	BCAA Metabolism		x	x
3-hydroxyisobutyrate	0.096	2.11E-10	1.39E-07	9.68	Amino Acid	BCAA Metabolism		x	x
beta-hydroxyisovalerate	0.144	9.71E-25	6.40E-22	24.01	Amino Acid	BCAA Metabolism		x	x
3-hydroxy-2-ethylpropionate	0.165	4.07E-35	2.68E-32	34.39	Amino Acid	BCAA Metabolism		x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
N-acetylleucine	0.179	8.51E-38	5.61E-35	37.07	Amino Acid	BCAA Metabolism	x	x	x
methylsuccinate	0.180	3.39E-31	2.23E-28	30.47	Amino Acid	BCAA Metabolism		x	x
ethylmalonate	0.207	6.54E-49	4.31E-46	48.18	Amino Acid	BCAA Metabolism		x	x
N-acetylvaline	0.227	7.90E-102	5.21E-99	101.10	Amino Acid	BCAA Metabolism		x	x
2,3-dihydroxy-2-methylbutyrate	0.277	2.67E-70	1.76E-67	69.57	Amino Acid	BCAA Metabolism	x	x	x
isobutyrylglycine (C4)	0.286	3.10E-64	2.05E-61	63.51	Amino Acid	BCAA Metabolism	x	x	x
isovalerylglycine	0.292	1.07E-52	7.04E-50	51.97	Amino Acid	BCAA Metabolism	x	x	x
3-methylglutaconate	0.379	4.60E-216	3.03E-213	215.34	Amino Acid	BCAA Metabolism	x	x	x
alpha-hydroxyisovalerate	0.084	2.00E-08	1.32E-05	7.70	Amino Acid	BCAA Metabolism	x	x	x
propionylglycine (C3)	0.097	9.32E-09	6.14E-06	8.03	Lipid	BCAA Metabolism	x	x	x
methylmalonate (MMA)	0.217	8.40E-37	5.53E-34	36.08	Lipid	BCAA Metabolism	x	x	x
6-oxopiperidine-2-carboxylate	0.125	7.50E-13	4.94E-10	12.12	Amino Acid	Lysine Metabolism		x	x
N6-acetyllysine	0.143	2.87E-31	1.89E-28	30.54	Amino Acid	Lysine Metabolism	x	x	x
N,N,N-trimethyl-5-aminovalerate	0.176	2.58E-46	1.70E-43	45.59	Amino Acid	Lysine Metabolism	x	x	x
N6,N6,N6-trimethyllysine	0.217	2.47E-61	1.63E-58	60.61	Amino Acid	Lysine Metabolism		x	x
glutaroylcarnitine (C5)	0.296	4.24E-118	2.79E-115	117.37	Amino Acid	Lysine Metabolism		x	x
5-(galactosylhydroxy)-L-lysine	0.333	1.02E-145	6.71E-143	144.99	Amino Acid	Lysine Metabolism	x	x	x
methionine sulfoxide	0.125	2.71E-16	1.79E-13	15.57	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism		x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
N-acetylmethionine	0.142	1.17E-15	7.73E-13	14.93	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	x	x	x
cysteine	0.154	1.50E-29	9.88E-27	28.82	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism		x	x
N-formylmethionine	0.173	2.16E-46	1.42E-43	45.67	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	x	x	x
cystathionine	0.228	3.09E-37	2.04E-34	36.51	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	x	x	x
S-adenosylhomocysteine (SAH)	0.251	6.40E-67	4.22E-64	66.19	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	x	x	x
N-acetyltaurine	0.269	5.06E-100	3.33E-97	99.30	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism		x	x
lanthionine	0.326	4.43E-92	2.92E-89	91.35	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	x	x	x
5-methylthioribose	0.416	1.76E-205	1.16E-202	204.76	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	x	x	x
phenylacetate	0.091	7.95E-06	5.24E-03	5.10	Amino Acid	Phenylalanine Metabolism	x		x
phenyllactate (PLA)	0.163	1.51E-23	9.97E-21	22.82	Amino Acid	Phenylalanine Metabolism	x	x	x
4-hydroxyphenylacetate	0.297	1.19E-42	7.86E-40	41.92	Amino Acid	Phenylalanine Metabolism	x	x	x
N-acetylphenylalanine	0.302	4.99E-72	3.29E-69	71.30	Amino Acid	Phenylalanine Metabolism	x	x	x
(N(1) + N(8))-acetylspermidine	0.169	8.44E-27	5.56E-24	26.07	Amino Acid	Polyamine Metabolism	x	x	x
5-methylthioadenosine (MTA)	0.178	8.83E-33	5.82E-30	32.05	Amino Acid	Polyamine Metabolism	x	x	x
N1,N12-diacetylspermine	0.230	9.88E-36	6.51E-33	35.01	Amino Acid	Polyamine Metabolism	x	x	x
acisoga	0.269	7.47E-62	4.92E-59	61.13	Amino Acid	Polyamine Metabolism		x	x
4-acetamidobutanoate	0.316	5.97E-119	3.94E-116	118.22	Amino Acid	Polyamine Metabolism		x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
N-acetyl-isoputreanine*	0.322	2.29E-118	1.51E-115	117.64	Amino Acid	Polyamine Metabolism	x	x	x
indole-3-carboxylate	0.072	4.48E-06	2.95E-03	5.35	Amino Acid	Tryptophan Metabolism	x	x	x
kynurenine	0.123	2.15E-22	1.42E-19	21.67	Amino Acid	Tryptophan Metabolism	x	x	x
indolelactate	0.162	5.02E-27	3.31E-24	26.30	Amino Acid	Tryptophan Metabolism	x	x	x
indoleacetate	0.167	1.09E-19	7.21E-17	18.96	Amino Acid	Tryptophan Metabolism	x	x	x
N-formylanthranilic acid	0.190	2.31E-33	1.52E-30	32.64	Amino Acid	Tryptophan Metabolism	x	x	x
xanthurenate	0.218	1.30E-25	8.59E-23	24.88	Amino Acid	Tryptophan Metabolism	x	x	x
picolinate	0.221	6.31E-33	4.16E-30	32.20	Amino Acid	Tryptophan Metabolism	x	x	x
3-indoxyl sulfate	0.261	1.65E-40	1.09E-37	39.78	Amino Acid	Tryptophan Metabolism		x	x
kynurenate	0.300	5.26E-60	3.46E-57	59.28	Amino Acid	Tryptophan Metabolism		x	x
C-glycosyltryptophan	0.318	9.15E-207	6.03E-204	206.04	Amino Acid	Tryptophan Metabolism	x	x	x
N-acetylkynurenine (2)	0.342	1.71E-54	1.13E-51	53.77	Amino Acid	Tryptophan Metabolism	x	x	x
indoleacetylglutamine	0.357	6.66E-72	4.39E-69	71.18	Amino Acid	Tryptophan Metabolism	x	x	x
N-acetyltryptophan	0.374	1.91E-12	1.26E-09	11.72	Amino Acid	Tryptophan Metabolism	x	x	x
N-formylphenylalanine	0.101	1.20E-06	7.91E-04	5.92	Amino Acid	Tyrosine Metabolism	x	x	x
dopamine 3-O-sulfate	0.130	1.33E-12	8.75E-10	11.88	Amino Acid	Tyrosine Metabolism	x	x	x
4-methoxyphenol sulfate	0.147	2.96E-13	1.95E-10	12.53	Amino Acid	Tyrosine Metabolism		x	x
3-methoxytyramine sulfate	0.169	4.25E-17	2.80E-14	16.37	Amino Acid	Tyrosine Metabolism		x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
3-(4-hydroxyphenyl)lactate (HPLA)	0.188	1.17E-38	7.70E-36	37.93	Amino Acid	Tyrosine Metabolism	x	x	x
phenol sulfate	0.205	1.68E-19	1.11E-16	18.78	Amino Acid	Tyrosine Metabolism		x	x
2-hydroxyphenylacetate	0.261	1.38E-48	9.07E-46	47.86	Amino Acid	Tyrosine Metabolism		x	x
4-hydroxyphenylacetatoylcarnitine	0.291	1.46E-33	9.63E-31	32.84	Amino Acid	Tyrosine Metabolism	x	x	x
homovanillate (HVA)	0.308	1.33E-88	8.80E-86	87.87	Amino Acid	Tyrosine Metabolism	x	x	x
N-acetyltyrosine	0.311	4.25E-65	2.80E-62	64.37	Amino Acid	Tyrosine Metabolism		x	x
p-cresol glucuronide*	0.324	4.19E-45	2.76E-42	44.38	Amino Acid	Tyrosine Metabolism		x	x
vanillylmandelate (VMA)	0.337	7.41E-85	4.89E-82	84.13	Amino Acid	Tyrosine Metabolism	x	x	x
vanillactate	0.354	5.65E-122	3.72E-119	121.25	Amino Acid	Tyrosine Metabolism	x	x	x
homovanillate sulfate	0.408	4.20E-103	2.77E-100	102.38	Amino Acid	Tyrosine Metabolism	x	x	x
N-delta-acetylornithine	0.079	1.19E-06	7.85E-04	5.92	Amino Acid	Urea cycle; Arginine and Proline Metabolism	x	x	x
2-oxoarginine*	0.108	1.25E-10	8.25E-08	9.90	Amino Acid	Urea cycle; Arginine and Proline Metabolism		x	x
N-acetylarginine	0.131	8.32E-19	5.48E-16	18.08	Amino Acid	Urea cycle; Arginine and Proline Metabolism		x	x
argininate*	0.179	5.71E-32	3.76E-29	31.24	Amino Acid	Urea cycle; Arginine and Proline Metabolism		x	x
N-acetylproline	0.195	2.97E-29	1.96E-26	28.53	Amino Acid	Urea cycle; Arginine and Proline Metabolism		x	x
prolylhydroxyproline	0.198	1.01E-59	6.67E-57	59.00	Amino Acid	Urea cycle; Arginine and Proline Metabolism		x	x
homocitrulline	0.234	1.15E-58	7.55E-56	57.94	Amino Acid	Urea cycle; Arginine and Proline Metabolism		x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
N2,N5-diacetylornithine	0.235	4.71E-32	3.10E-29	31.33	Amino Acid	Urea cycle; Arginine and Proline Metabolism		x	x
urea	0.288	3.49E-122	2.30E-119	121.46	Amino Acid	Urea cycle; Arginine and Proline Metabolism	x	x	x
N-acetylcitrulline	0.293	1.46E-47	9.65E-45	46.83	Amino Acid	Urea cycle; Arginine and Proline Metabolism		x	x
N6-carboxymethyllysine	0.295	3.23E-75	2.13E-72	74.49	Carbohydrate	Advanced Glycation End-product	x	x	x
N-acetylglucosaminylasparagine	0.209	3.32E-40	2.19E-37	39.48	Carbohydrate	Aminosugar Metabolism	x	x	x
glucuronate	0.285	4.42E-68	2.91E-65	67.35	Carbohydrate	Aminosugar Metabolism		x	x
N-acetylneuraminate	0.313	7.30E-199	4.81E-196	198.14	Carbohydrate	Aminosugar Metabolism	x	x	x
erythronate*	0.330	4.67E-277	3.08E-274	276.33	Carbohydrate	Aminosugar Metabolism	x	x	x
sucrose	0.272	5.11E-29	3.37E-26	28.29	Carbohydrate	Disaccharides and Oligosaccharides	x	x	x
galactonate	0.112	5.74E-07	3.78E-04	6.24	Carbohydrate	Fructose, Mannose and Galactose Metabolism		x	x
mannitol/sorbitol	0.246	3.52E-20	2.32E-17	19.45	Carbohydrate	Fructose, Mannose and Galactose Metabolism	x	x	x
maltotriose	0.110	2.91E-08	1.92E-05	7.54	Carbohydrate	Glycogen Metabolism		x	x
maltose	0.153	6.37E-13	4.20E-10	12.20	Carbohydrate	Glycogen Metabolism	x	x	x
maltotetraose	0.179	2.82E-19	1.86E-16	18.55	Carbohydrate	Glycogen Metabolism		x	x
xylose	0.128	2.73E-17	1.80E-14	16.56	Carbohydrate	Pentose Metabolism		x	x
sedoheptulose	0.144	4.82E-18	3.18E-15	17.32	Carbohydrate	Pentose Metabolism		x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
Arabinose	0.161	2.27E-27	1.49E-24	26.64	Carbohydrate	Pentose Metabolism		x	x
arabitol/xylitol	0.241	4.50E-59	2.97E-56	58.35	Carbohydrate	Pentose Metabolism		x	x
ribulonate/xylulonate*	0.273	3.15E-87	2.08E-84	86.50	Carbohydrate	Pentose Metabolism		x	x
ribonate (ribonolactone)	0.293	9.65E-151	6.36E-148	150.02	Carbohydrate	Pentose Metabolism	x	x	x
arabonate/xylonate	0.356	3.33E-238	2.20E-235	237.48	Carbohydrate	Pentose Metabolism	x	x	x
threonate	0.186	5.19E-42	3.42E-39	41.28	Cofactors and Vitamins	Ascorbate and Aldarate Metabolism		x	x
gulonate*	0.372	2.76E-108	1.82E-105	107.56	Cofactors and Vitamins	Ascorbate and Aldarate Metabolism		x	x
1-methylnicotinamide	0.077	6.34E-05	4.18E-02	4.20	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism		x	
N1-Methyl-2-pyridone-5-carboxamide	0.200	1.02E-37	6.69E-35	36.99	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism	x	x	x
nicotinamide riboside	0.210	1.57E-35	1.04E-32	34.80	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism		x	x
trigonelline (N'-methylnicotinate)	0.217	5.20E-16	3.43E-13	15.28	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism	x	x	x
quinolinate	0.390	1.48E-116	9.77E-114	115.83	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism		x	x
pantothenate (Vitamin B5)	0.107	8.21E-16	5.41E-13	15.09	Cofactors and Vitamins	Pantothenate and CoA Metabolism	x	x	x
alpha-CEHC sulfate	0.194	8.65E-16	5.70E-13	15.06	Cofactors and Vitamins	Tocopherol Metabolism	x	x	x
alpha-CEHC glucuronide*	0.207	2.79E-22	1.84E-19	21.55	Cofactors and Vitamins	Tocopherol Metabolism		x	x
gamma-CEHC glucuronide*	0.357	3.01E-77	1.98E-74	76.52	Cofactors and Vitamins	Tocopherol Metabolism		x	x
retinol (Vitamin A)	0.070	2.19E-06	1.44E-03	5.66	Cofactors and Vitamins	Vitamin A Metabolism	x	x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
pyridoxate	0.278	8.31E-35	5.48E-32	34.08	Cofactors and Vitamins	Vitamin B6 Metabolism	x	x	x
succinylcarnitine (C4)	0.262	2.46E-85	1.62E-82	84.61	Energy	TCA Cycle		x	x
2-methylcitrate/homocitrate	0.285	1.30E-60	8.57E-58	59.89	Energy	TCA Cycle	x	x	x
5alpha-androstan-3beta,17beta-diol disulfate	0.069	4.35E-06	2.87E-03	5.36	Lipid	Androgenic Steroids	x	x	x
5alpha-androstan-3alpha,17beta-diol monosulfate (1)	0.107	4.98E-06	3.28E-03	5.30	Lipid	Androgenic Steroids	x		x
androstenediol (3beta,17beta) disulfate (2)	0.112	1.92E-12	1.26E-09	11.72	Lipid	Androgenic Steroids		x	x
5alpha-androstan-3beta,17alpha-diol disulfate	0.117	1.74E-09	1.14E-06	8.76	Lipid	Androgenic Steroids		x	x
andro steroid monosulfate C19H28O6S (1)*	0.130	1.79E-09	1.18E-06	8.75	Lipid	Androgenic Steroids		x	x
etiocholanolone glucuronide	0.308	1.08E-54	7.14E-52	53.97	Lipid	Androgenic Steroids		x	x
androsterone glucuronide	0.330	4.11E-71	2.71E-68	70.39	Lipid	Androgenic Steroids		x	x
deoxycarnitine	0.148	6.24E-31	4.11E-28	30.20	Lipid	Carnitine Metabolism	x	x	x
N-behenoyl-sphingadienine (d18:2/22:0)*	0.064	7.86E-06	5.18E-03	5.10	Lipid	Ceramides	x	x	x
N-palmitoyl-heptadecasphingosine (d17:1/16:0)*	0.067	4.17E-06	2.75E-03	5.38	Lipid	Ceramides		x	x
N-palmitoyl-sphingosine (d18:1/16:0)	0.073	2.83E-07	1.87E-04	6.55	Lipid	Ceramides		x	x
oleoyl-oleoyl-glycerol (18:1/18:1) [1]*	0.063	3.68E-06	2.43E-03	5.43	Lipid	Diacylglycerol		x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
oleoyl-oleoyl-glycerol (18:1/18:1) [2]*	0.074	2.80E-07	1.85E-04	6.55	Lipid	Diacylglycerol		x	x
hexanoylglutamine	0.234	2.16E-39	1.42E-36	38.67	Lipid	Fatty Acid Metabolism (Acyl Glutamine)		x	x
propionylcarnitine (C3)	0.156	2.22E-21	1.46E-18	20.65	Lipid	Acylcarnitine		x	x
butyrylcarnitine (C4)	0.095	6.81E-08	4.48E-05	7.17	Lipid	Acylcarnitine	x	x	x
isobutyrylcarnitine (C4)	0.255	1.26E-39	8.28E-37	38.90	Lipid	Acylcarnitine		x	x
2-methylbutyroylcarnitine (C5)	0.276	3.84E-64	2.53E-61	63.42	Lipid	Acylcarnitine		x	x
isovalerylcarnitine (C5)	0.187	5.56E-28	3.67E-25	27.25	Lipid	Acylcarnitine		x	x
tiglyl carnitine (C5)	0.293	3.18E-88	2.09E-85	87.50	Lipid	Acylcarnitine	x	x	x
3-methylglutarylcarnitine (C6-DC)	0.407	4.18E-170	2.75E-167	169.38	Lipid	Acylcarnitine		x	x
myristoylcarnitine (C14)	0.068	1.23E-05	8.09E-03	4.91	Lipid	Acylcarnitine		x	x
myristoleoylcarnitine (C14:1)*	0.070	3.76E-05	2.48E-02	4.43	Lipid	Acylcarnitine			x
3-hydroxyoleoylcarnitine (C18:1-OH)	0.080	1.87E-06	1.23E-03	5.73	Lipid	Acylcarnitine	x	x	x
laurylcarnitine (C12)	0.112	1.20E-12	7.92E-10	11.92	Lipid	Acylcarnitine		x	x
octadecanedioylcarnitine (C18-DC)*	0.121	8.20E-13	5.40E-10	12.09	Lipid	Acylcarnitine	x	x	x
3-hydroxybutyrylcarnitine (1) (C4-OH)	0.134	4.37E-09	2.88E-06	8.36	Lipid	Acylcarnitine	x	x	x
5-dodecenoylcarnitine (C12:1)	0.137	2.17E-14	1.43E-11	13.66	Lipid	Acylcarnitine		x	x
acetylcarnitine (C2)	0.148	2.36E-26	1.55E-23	25.63	Lipid	Acylcarnitine		x	x
decanoylcarnitine (C10)	0.155	6.77E-23	4.46E-20	22.17	Lipid	Acylcarnitine		x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
hexanoylcarnitine (C6)	0.170	1.64E-26	1.08E-23	25.78	Lipid	Acylcarnitine		x	x
octanoylcarnitine (C8)	0.178	9.66E-30	6.36E-27	29.02	Lipid	Acylcarnitine		x	x
octadecenedioylcarnitine (C18:1-DC)*	0.180	2.50E-19	1.65E-16	18.60	Lipid	Acylcarnitine	x	x	x
cis-4-decenoylcarnitine (C10:1)	0.188	3.66E-36	2.41E-33	35.44	Lipid	Acylcarnitine	x	x	x
3-hydroxybutyrylcarnitine (2) (C4-OH)	0.210	4.14E-38	2.73E-35	37.38	Lipid	Acylcarnitine		x	x
adipoylcarnitine (C6-DC)	0.333	2.00E-99	1.32E-96	98.70	Lipid	Acylcarnitine	x	x	x
suberoylcarnitine (C8-DC)	0.336	1.83E-78	1.20E-75	77.74	Lipid	Acylcarnitine	x	x	x
pimeloylcarnitine/3-methyladipoylcarnitine (C7-DC)	0.361	7.15E-143	4.71E-140	142.15	Lipid	Acylcarnitine	x	x	x
hexanoylglycine (C6)	0.100	6.26E-08	4.13E-05	7.20	Lipid	Fatty Acid Metabolism(Acyl Glycine)	x		x
trans-2-hexenoylglycine	0.202	1.09E-28	7.18E-26	27.96	Lipid	Fatty Acid Metabolism(Acyl Glycine)	x	x	x
malonylcarnitine	0.226	1.08E-59	7.09E-57	58.97	Lipid	Fatty Acid Synthesis	x	x	x
2-methylmalonylcarnitine (C4-DC)	0.392	1.86E-164	1.23E-161	163.73	Lipid	Fatty Acid Synthesis	x	x	x
N-acetyl-2-aminooctanoate*	0.122	1.71E-10	1.13E-07	9.77	Lipid	Fatty Acid, Amino	x	x	x
azelate (nonanedioate; C9)	0.087	4.42E-08	2.91E-05	7.35	Lipid	Fatty Acid, Dicarboxylate		x	x
maleate	0.103	6.08E-05	4.01E-02	4.22	Lipid	Fatty Acid, Dicarboxylate		x	
adipate	0.133	2.63E-13	1.73E-10	12.58	Lipid	Fatty Acid, Dicarboxylate	x	x	x
3-carboxy-4-methyl-5-pentyl-2-furanpropionate (3-CMPFP)	0.153	1.14E-24	7.53E-22	23.94	Lipid	Fatty Acid, Dicarboxylate	x	x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
suberate (C8-DC)	0.259	4.13E-64	2.72E-61	63.38	Lipid	Fatty Acid, Dicarboxylate		x	x
2-hydroxyadipate	0.263	1.44E-43	9.51E-41	42.84	Lipid	Fatty Acid, Dicarboxylate	x	x	x
heptenedioate (C7:1-DC)*	0.320	1.95E-82	1.28E-79	81.71	Lipid	Fatty Acid, Dicarboxylate	x	x	x
3-hydroxyadipate*	0.321	5.61E-73	3.70E-70	72.25	Lipid	Fatty Acid, Dicarboxylate	x	x	x
3-methyladipate	0.356	8.50E-123	5.60E-120	122.07	Lipid	Fatty Acid, Dicarboxylate		x	x
3-hydroxyhexanoate	0.098	6.53E-10	4.31E-07	9.18	Lipid	Fatty Acid, Monohydroxy	x	x	x
5-hydroxyhexanoate	0.151	3.20E-27	2.11E-24	26.50	Lipid	Fatty Acid, Monohydroxy	x	x	x
myo-inositol	0.231	1.36E-45	8.97E-43	44.87	Lipid	Inositol Metabolism	x	x	x
3-hydroxy-3-methylglutarate	0.225	1.18E-54	7.78E-52	53.93	Lipid	Mevalonate Metabolism		x	x
1,2-dilinoleoyl-GPE (18:2/18:2)*	0.083	3.70E-06	2.44E-03	5.43	Lipid	Phosphatidylethanolamine		x	x
1-palmitoyl-2-docosahexaenoyl-GPE (16:0/22:6)*	0.084	2.10E-11	1.39E-08	10.68	Lipid	Phosphatidylethanolamine		x	x
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	0.089	1.22E-10	8.07E-08	9.91	Lipid	Phosphatidylethanolamine		x	x
1-stearoyl-2-linoleoyl-GPE (18:0/18:2)*	0.089	3.40E-11	2.24E-08	10.47	Lipid	Phosphatidylethanolamine		x	x
1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)*	0.094	2.17E-13	1.43E-10	12.66	Lipid	Phosphatidylethanolamine		x	x
1-oleoyl-2-arachidonoyl-GPE (18:1/20:4)*	0.105	1.18E-15	7.80E-13	14.93	Lipid	Phosphatidylethanolamine		x	x
1-oleoyl-2-linoleoyl-GPE (18:1/18:2)*	0.105	1.28E-12	8.44E-10	11.89	Lipid	Phosphatidylethanolamine		x	x
1-stearoyl-2-oleoyl-GPE (18:0/18:1)	0.107	1.28E-11	8.40E-09	10.89	Lipid	Phosphatidylethanolamine		x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	0.110	1.00E-14	6.62E-12	14.00	Lipid	Phosphatidylethanolamine		x	x
1-oleoyl-2-docosahexaenoyl-GPE (18:1/22:6)*	0.111	3.83E-13	2.52E-10	12.42	Lipid	Phosphatidylethanolamine	x	x	x
trimethylamine N-oxide	0.250	2.16E-39	1.43E-36	38.67	Lipid	Phospholipid Metabolism		x	x
pregnen-diol disulfate*	0.072	1.57E-05	1.04E-02	4.80	Lipid	Pregnenolone Steroids		x	x
21-hydroxypregnenolone disulfate	0.150	1.07E-16	7.02E-14	15.97	Lipid	Pregnenolone Steroids		x	x
glycochenodeoxycholate glucuronide (1)	0.101	2.44E-06	1.61E-03	5.61	Lipid	Primary Bile Acid Metabolism		x	x
glycochenodeoxycholate	0.104	9.05E-06	5.97E-03	5.04	Lipid	Primary Bile Acid Metabolism		x	x
glycocholate glucuronide (1)	0.105	3.48E-06	2.29E-03	5.46	Lipid	Primary Bile Acid Metabolism		x	x
taurocholate	0.118	1.23E-05	8.11E-03	4.91	Lipid	Primary Bile Acid Metabolism		x	x
glycochenodeoxycholate sulfate	0.133	4.96E-08	3.27E-05	7.30	Lipid	Primary Bile Acid Metabolism		x	x
taurochenodeoxycholate	0.137	1.08E-07	7.12E-05	6.97	Lipid	Primary Bile Acid Metabolism		x	x
glycocholate sulfate	0.139	3.35E-08	2.21E-05	7.48	Lipid	Primary Bile Acid Metabolism		x	x
5alpha-pregnan-3beta,20alpha-diol disulfate	0.122	3.84E-10	2.53E-07	9.42	Lipid	Progestin Steroids		x	x
5alpha-pregnan-diol disulfate	0.128	1.48E-07	9.77E-05	6.83	Lipid	Progestin Steroids		x	x
pregnanediol-3-glucuronide	0.194	2.14E-23	1.41E-20	22.67	Lipid	Progestin Steroids		x	x
glycocholenate sulfate*	0.086	1.30E-05	8.58E-03	4.89	Lipid	Secondary Bile Acid Metabolism		x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
taurocholenate sulfate*	0.108	4.23E-06	2.79E-03	5.37	Lipid	Secondary Bile Acid Metabolism		x	x
glycolithocholate sulfate*	0.113	1.32E-05	8.73E-03	4.88	Lipid	Secondary Bile Acid Metabolism		x	x
taurolithocholate 3-sulfate	0.128	7.16E-07	4.72E-04	6.15	Lipid	Secondary Bile Acid Metabolism		x	x
glycodeoxycholate sulfate	0.159	1.05E-08	6.93E-06	7.98	Lipid	Secondary Bile Acid Metabolism		x	x
urate	0.171	1.98E-42	1.30E-39	41.70	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	x	x	x
xanthosine	0.179	6.85E-23	4.51E-20	22.16	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	x	x	x
N1-methylinosine	0.338	1.67E-164	1.10E-161	163.78	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing		x	x
adenosine	0.124	5.88E-16	3.88E-13	15.23	Nucleotide	Purine Metabolism, Adenine containing	x	x	x
N6-succinyladenosine	0.347	2.59E-134	1.70E-131	133.59	Nucleotide	Purine Metabolism, Adenine containing	x	x	x
N6-carbamoylthreonyladenosine	0.384	3.81E-274	2.51E-271	273.42	Nucleotide	Purine Metabolism, Adenine containing	x	x	x
N2,N2-dimethylguanosine	0.282	1.33E-99	8.79E-97	98.87	Nucleotide	Purine Metabolism, Guanine containing	x	x	x
cytidine	0.208	4.79E-39	3.15E-36	38.32	Nucleotide	Pyrimidine Metabolism, Cytidine containing		x	x
orotate	0.124	2.81E-10	1.85E-07	9.55	Nucleotide	Pyrimidine Metabolism, Orotate containing	x	x	x
orotidine	0.343	2.19E-58	1.44E-55	57.66	Nucleotide	Pyrimidine Metabolism, Orotate containing	x	x	x
3-aminoisobutyrate	0.146	5.50E-18	3.62E-15	17.26	Nucleotide	Pyrimidine Metabolism, Thymine containing		x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
beta-alanine	0.097	9.53E-13	6.28E-10	12.02	Nucleotide	Pyrimidine Metabolism, Uracil containing		x	x
3-ureidopropionate	0.142	2.28E-14	1.50E-11	13.64	Nucleotide	Pyrimidine Metabolism, Uracil containing	x	x	x
N-acetyl-beta-alanine	0.152	1.07E-35	7.05E-33	34.97	Nucleotide	Pyrimidine Metabolism, Uracil containing	x	x	x
pseudouridine	0.289	1.10E-220	7.22E-218	219.96	Nucleotide	Pyrimidine Metabolism, Uracil containing	x	x	x
5,6-dihydrouridine	0.297	3.00E-168	1.98E-165	167.52	Nucleotide	Pyrimidine Metabolism, Uracil containing	x	x	x
glycine conjugate of C10H14O2 (1)*	0.203	9.61E-28	6.33E-25	27.02	Partially Characterized Molecules	Partially Characterized Molecules	x	x	x
glucuronide of C10H18O2 (7)*	0.263	6.98E-26	4.60E-23	25.16	Partially Characterized Molecules	Partially Characterized Molecules	x	x	x
phenylacetylglutamine	0.318	7.06E-82	4.66E-79	81.15	Peptide	Acetylated Peptides	x	x	x
phenylacetylmethionine	0.380	3.15E-78	2.07E-75	77.50	Peptide	Acetylated Peptides	x	x	x
phenylacetylglutamate	0.431	5.31E-104	3.50E-101	103.27	Peptide	Acetylated Peptides	x	x	x
4-hydroxyphenylacetylglutamine	0.440	1.73E-83	1.14E-80	82.76	Peptide	Acetylated Peptides	x	x	x
prolylglycine	0.218	2.37E-40	1.56E-37	39.62	Peptide	Dipeptide	x	x	x
1H-indole-7-acetic acid	0.123	1.06E-11	6.98E-09	10.97	Xenobiotics	Bacterial/Fungal	x	x	x
N-methylpipecolate	0.249	2.90E-45	1.91E-42	44.54	Xenobiotics	Bacterial/Fungal	x	x	x
3-methyl catechol sulfate (1)	0.142	8.98E-09	5.92E-06	8.05	Xenobiotics	Benzoate Metabolism	x	x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
methyl-4-hydroxybenzoate sulfate	0.156	1.49E-10	9.83E-08	9.83	Xenobiotics	Benzoate Metabolism	x	x	x
4-vinylphenol sulfate	0.164	7.73E-12	5.09E-09	11.11	Xenobiotics	Benzoate Metabolism	x	x	x
catechol sulfate	0.189	5.03E-13	3.31E-10	12.30	Xenobiotics	Benzoate Metabolism	x	x	x
3-hydroxyhippurate	0.192	1.82E-14	1.20E-11	13.74	Xenobiotics	Benzoate Metabolism	x	x	x
p-cresol sulfate	0.206	9.68E-24	6.38E-21	23.01	Xenobiotics	Benzoate Metabolism	x	x	x
4-methylcatechol sulfate	0.251	1.58E-24	1.04E-21	23.80	Xenobiotics	Benzoate Metabolism	x	x	x
3-methoxycatechol sulfate (1)	0.261	1.46E-36	9.64E-34	35.84	Xenobiotics	Benzoate Metabolism	x	x	x
4-methylguaiacol sulfate	0.312	3.29E-44	2.17E-41	43.48	Xenobiotics	Benzoate Metabolism	x	x	x
4-hydroxyhippurate	0.333	7.48E-56	4.93E-53	55.13	Xenobiotics	Benzoate Metabolism	x	x	x
hippurate	0.368	3.91E-61	2.58E-58	60.41	Xenobiotics	Benzoate Metabolism	x	x	x
guaiacol sulfate	0.396	9.35E-71	6.16E-68	70.03	Xenobiotics	Benzoate Metabolism	x	x	x
1,2,3-benzenetriol sulfate (2)	0.101	2.07E-05	1.37E-02	4.68	Xenobiotics	Chemical	x	x	x
3-hydroxypyridine sulfate	0.187	3.70E-09	2.44E-06	8.43	Xenobiotics	Chemical	x	x	x
ectoine	0.192	1.56E-23	1.03E-20	22.81	Xenobiotics	Chemical		x	x
sulfate*	0.250	2.17E-120	1.43E-117	119.66	Xenobiotics	Chemical	x	x	x
6-hydroxyindole sulfate	0.261	1.37E-40	9.02E-38	39.86	Xenobiotics	Chemical	x	x	x
2-aminophenol sulfate	0.265	1.97E-40	1.30E-37	39.71	Xenobiotics	Chemical	x	x	x
O-sulfo-L-tyrosine	0.358	3.40E-261	2.24E-258	260.47	Xenobiotics	Chemical		x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
salicyluric glucuronide*	0.176	4.77E-15	3.14E-12	14.32	Xenobiotics	Drug - Analgesics, Anesthetics	x	x	x
4-acetamidophenylglucuronide	0.196	1.47E-06	9.71E-04	5.83	Xenobiotics	Drug - Analgesics, Anesthetics		x	x
furosemide	0.296	8.06E-29	5.31E-26	28.09	Xenobiotics	Drug - Cardiovascular		x	x
metoclopramide	0.096	3.08E-05	2.03E-02	4.51	Xenobiotics	Drug - Gastrointestinal			x
S-carboxymethyl-L-cysteine	0.131	1.09E-10	7.19E-08	9.96	Xenobiotics	Drug - Other	x	x	x
hydroquinone sulfate	0.325	2.74E-64	1.80E-61	63.56	Xenobiotics	Drug - Topical Agents		x	x
umbelliferone sulfate	0.081	3.30E-05	2.18E-02	4.48	Xenobiotics	Food Component/Plant	x	x	x
eugenol sulfate	0.107	6.85E-06	4.52E-03	5.16	Xenobiotics	Food Component/Plant		x	x
homostachydrine*	0.150	2.38E-16	1.57E-13	15.62	Xenobiotics	Food Component/Plant		x	x
stachydrine	0.160	9.57E-12	6.31E-09	11.02	Xenobiotics	Food Component/Plant	x	x	x
tartarate	0.161	9.48E-11	6.25E-08	10.02	Xenobiotics	Food Component/Plant		x	x
gluconate	0.200	1.54E-12	1.02E-09	11.81	Xenobiotics	Food Component/Plant	x	x	x
cinnamoylglycine	0.201	5.23E-20	3.45E-17	19.28	Xenobiotics	Food Component/Plant		x	x
indolin-2-one	0.210	3.52E-35	2.32E-32	34.45	Xenobiotics	Food Component/Plant		x	x
3-hydroxystachydrine*	0.225	9.87E-22	6.50E-19	21.01	Xenobiotics	Food Component/Plant	x	x	x
saccharin	0.242	1.32E-21	8.72E-19	20.88	Xenobiotics	Food Component/Plant		x	x
2-keto-3-deoxy-gluconate	0.276	2.29E-91	1.51E-88	90.64	Xenobiotics	Food Component/Plant	x	x	x
quinate	0.281	1.79E-23	1.18E-20	22.75	Xenobiotics	Food Component/Plant	x	x	x

Supplemental Table 3. Metabolites significantly increased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
2,3-dihydroxyisovalerate	0.320	2.41E-58	1.59E-55	57.62	Xenobiotics	Food Component/Plant	x	x	x
methyl indole-3-acetate	0.342	9.70E-76	6.39E-73	75.01	Xenobiotics	Food Component/Plant	x	x	x
N-(2-furoyl)glycine	0.354	4.45E-52	2.93E-49	51.35	Xenobiotics	Food Component/Plant		x	x
erythritol	0.355	4.91E-280	3.24E-277	279.31	Xenobiotics	Food Component/Plant		x	x
7-methylxanthine	0.106	1.83E-06	1.20E-03	5.74	Xenobiotics	Xanthine Metabolism			x
3-methylxanthine	0.115	4.62E-07	3.05E-04	6.34	Xenobiotics	Xanthine Metabolism		x	x
1,3-dimethylurate	0.132	2.15E-06	1.41E-03	5.67	Xenobiotics	Xanthine Metabolism	x	x	x
7-methylurate	0.134	7.71E-15	5.08E-12	14.11	Xenobiotics	Xanthine Metabolism	x	x	x
1,3,7-trimethylurate	0.142	4.97E-12	3.28E-09	11.30	Xenobiotics	Xanthine Metabolism	x	x	x
1,7-dimethylurate	0.143	1.13E-10	7.46E-08	9.95	Xenobiotics	Xanthine Metabolism	x	x	x
5-acetylamino-6-amino-3-methyluracil	0.194	4.62E-18	3.04E-15	17.34	Xenobiotics	Xanthine Metabolism	x	x	x
1-methylurate	0.384	4.49E-133	2.96E-130	132.35	Xenobiotics	Xanthine Metabolism		x	x

Note: Using longitudinal data (day 0, 3, and 7), the association between relative quantitation of each individual metabolite noted above and Creatinine (Cr) levels over time were estimated utilizing linear mixed-effects models adjusting for age, sex, baseline 25(OH)D, an absolute increase in 25(OH)D, Simplified Acute Physiology Score (SAPS) II, plasma day, admission diagnosis and individual subjects (as the random-intercept). Known uremic solutes were reported in the previously longest published list of uremic solutes (15). Adding mixed-effect model adjusted serum procalcitonin levels besides age, sex, baseline 25(OH)D, an absolute increase in 25(OH)D, Simplified Acute Physiology Score (SAPS) II, plasma day, admission diagnosis and individual subjects (as the random-intercept). All significant mixed-effects associations have P-value $<7.59 \times 10^{-5}$. For the Acylcarnitines sub pathway: a capital C is followed by the number of carbons within the fatty acyl group attached to the carnitine. A colon followed by a number is one or more unsaturated

carbons in the acylcarnitine ester (i.e. C10:1 is a monounsaturated C10 acylcarnitine). OH following the carbon number is a hydroxylic acylcarnitine. DC following the carbon number is a dicarboxylic acylcarnitine. GPE is glycerophosphoethanolamine.

Supplemental Table 4. Metabolites significantly decreased with increased Creatinine over days 0-7

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
2-aminobutyrate	-0.075	1.79E-08	1.18E-05	7.75	Amino Acid	Glutathione Metabolism		x	x
4-methyl-2-oxopentanoate	-0.091	2.23E-12	1.47E-09	11.65	Amino Acid	BCAA Metabolism		x	x
S-methylcysteine	-0.071	1.06E-07	6.96E-05	6.98	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism		x	x
serotonin	-0.107	1.06E-06	6.98E-04	5.98	Amino Acid	Tryptophan Metabolism		x	x
thyroxine	-0.093	5.08E-13	3.35E-10	12.29	Amino Acid	Tyrosine Metabolism		x	x
fructose	-0.067	3.97E-07	2.62E-04	6.40	Carbohydrate	Fructose, Mannose and Galactose Metabolism		x	x
bilirubin (E,E)*	-0.074	4.60E-06	3.03E-03	5.34	Cofactors and Vitamins	Hemoglobin and Porphyrin Metabolism		x	x
arachidonoylcholine	-0.162	3.04E-18	2.00E-15	17.52	Lipid	Acyl Choline		x	x
dihomo-linolenoyl-choline	-0.156	3.07E-16	2.02E-13	15.51	Lipid	Acyl Choline		x	x
linoleoylcholine*	-0.154	4.89E-16	3.22E-13	15.31	Lipid	Acyl Choline		x	x
palmitoylcholine	-0.150	1.10E-15	7.25E-13	14.96	Lipid	Acyl Choline		x	x
stearoylcholine*	-0.150	1.27E-13	8.34E-11	12.90	Lipid	Acyl Choline		x	x
oleoylcholine	-0.143	1.01E-13	6.66E-11	13.00	Lipid	Acyl Choline		x	x
docosahexaenoylcholine	-0.142	1.86E-14	1.22E-11	13.73	Lipid	Acyl Choline		x	x
2-aminooctanoate	-0.100	1.94E-08	1.28E-05	7.71	Lipid	Fatty Acid, Amino		x	x
hydroxy-CMPF*	-0.067	6.94E-07	4.57E-04	6.16	Lipid	Fatty Acid, Dicarboxylate	x	x	x
1-arachidonoyl-GPC* (20:4)*	-0.105	6.57E-16	4.33E-13	15.18	Lipid	Lysophospholipid		x	x
1-palmitoleoyl-GPC* (16:1)*	-0.083	2.53E-09	1.67E-06	8.60	Lipid	Lysophospholipid		x	x

Supplemental Table 4. Metabolites significantly decreased with increased Creatinine over days 0-7 (Continued)

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway	Known Uremic Solutes (15)	Significant in the cohort without hemodialysis	Significant in adding mixed effect model
1-linolenoyl-GPC (18:3)*	-0.081	1.69E-07	1.12E-04	6.77	Lipid	Lysophospholipid		x	x
2-palmitoyl-GPC* (16:0)*	-0.065	3.74E-06	2.47E-03	5.43	Lipid	Lysophospholipid		x	x
1-cerotoyl-GPC (26:0)*	-0.060	2.52E-05	1.66E-02	4.60	Lipid	Lysophospholipid			x
1-(1-enyl-palmitoyl)-GPC (P-16:0)*	-0.112	2.05E-14	1.35E-11	13.69	Lipid	Lysoplasmalogen		x	x
1-(1-enyl-stearoyl)-GPE (P-18:0)*	-0.068	6.74E-06	4.44E-03	5.17	Lipid	Lysoplasmalogen		x	x
1-(1-enyl-oleoyl)-GPE (P-18:1)*	-0.068	3.05E-05	2.01E-02	4.52	Lipid	Lysoplasmalogen		x	
1-(1-enyl-palmitoyl)-GPE (P-16:0)*	-0.067	7.65E-06	5.04E-03	5.12	Lipid	Lysoplasmalogen		x	x
glycerophosphorylcholine (GPC)	-0.117	3.09E-17	2.04E-14	16.51	Lipid	Phospholipid Metabolism		x	x
3-methylcytidine	-0.069	5.75E-06	3.79E-03	5.24	Nucleotide	Pyrimidine Metabolism, Cytidine containing			x
uridine	-0.134	4.56E-30	3.01E-27	29.34	Nucleotide	Pyrimidine Metabolism, Uracil containing		x	x
gamma-glutamyl-2-aminobutyrate	-0.119	1.79E-12	1.18E-09	11.75	Peptide	Gamma-glutamyl Amino Acid		x	x
gamma-glutamylcitrulline*	-0.099	4.68E-09	3.09E-06	8.33	Peptide	Gamma-glutamyl Amino Acid		x	x
gamma-glutamylglutamine	-0.093	1.25E-09	8.25E-07	8.90	Peptide	Gamma-glutamyl Amino Acid		x	x
gamma-glutamylmethionine	-0.088	7.36E-08	4.85E-05	7.13	Peptide	Gamma-glutamyl Amino Acid		x	x
gamma-glutamylthreonine	-0.069	2.86E-06	1.88E-03	5.54	Peptide	Gamma-glutamyl Amino Acid	x	x	x
thioproline	-0.058	7.29E-06	4.80E-03	5.14	Xenobiotics	Chemical	x	x	x

Note: Using longitudinal data (day 0, 3, and 7), the association between relative quantitation of each individual metabolite noted above and Creatinine (Cr) levels over time were estimated utilizing linear mixed-effects models adjusting for age, sex, baseline 25(OH)D, an absolute increase in 25(OH)D, Simplified Acute Physiology Score (SAPS) II, plasma day, admission diagnosis and individual subjects (as the random-intercept). Known uremic solutes were reported in the previously longest published list of uremic solutes(15). Adding mixed-effect model adjusted serum procalcitonin levels besides age, sex, baseline 25(OH)D, an absolute increase in 25(OH)D, Simplified Acute Physiology Score (SAPS) II, plasma day, admission diagnosis and individual subjects (as the random-intercept). All significant mixed-effects associations have P-value $<7.59 \times 10^{-5}$. GPC is glycerophosphorylcholine; GPE is glycerophosphoethanolamine.

Supplemental Table 5. Metabolite significantly changed with increased Creatinine over days 0-7 among the mixed effect model adjusting Procalcitonin

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-$\log_{10}$p	Super Pathway	Sub Pathway
N-methylhydroxyproline	0.116	6.60E-05	4.35E-02	4.18	Amino Acid	Urea cycle; Arginine and Proline Metabolism

Note: Using longitudinal data (day 0, 3, and 7), the association between relative quantitation of each individual metabolite noted above and Creatinine (Cr) levels over time were estimated utilizing linear mixed-effects models adjusting for age, sex, baseline 25(OH)D, an absolute increase in 25(OH)D, Simplified Acute Physiology Score (SAPS) II, plasma day, admission diagnosis, plasma procalcitonin levels and individual subjects (as the random-intercept). The significant mixed-effects associations have P-value $<7.59 \times 10^{-5}$.

Supplemental Table 6. Metabolites significantly changed with increased Creatinine over days 0-7 among the cohort without ESRD subjects at baseline

Metabolite	β Coefficient of Cr	P-value	Bonferroni Corrected P-value	-log$_{10}$p	Super Pathway	Sub Pathway
ceramide (d18:1/17:0, d17:1/18:0)*	0.079	1.23E-05	8.11E-03	4.91	Lipid	Ceramides
N-stearoyl-sphingosine (d18:1/18:0)*	0.078	6.33E-06	4.17E-03	5.20	Lipid	Ceramides
oleoyl-linoleoyl-glycerol (18:1/18:2) [2]	0.065	2.10E-05	1.39E-02	4.68	Lipid	Diacylglycerol
palmitoyl-oleoyl-glycerol (16:0/18:1) [1]*	0.067	3.16E-05	2.08E-02	4.50	Lipid	Diacylglycerol
palmitoyl-oleoyl-glycerol (16:0/18:1) [2]*	0.076	3.38E-06	2.23E-03	5.47	Lipid	Diacylglycerol
N-palmitoyl-sphinganine (d18:0/16:0)	0.079	8.05E-06	5.31E-03	5.09	Lipid	Dihydroceramides
1-linoleoyl-GPG (18:2)*	0.066	6.96E-05	4.59E-02	4.16	Lipid	Lysophospholipid
1-oleoyl-GPG (18:1)*	0.086	1.85E-05	1.22E-02	4.73	Lipid	Lysophospholipid
1-myristoyl-2-palmitoyl-GPC (14:0/16:0)	0.065	1.39E-05	9.16E-03	4.86	Lipid	Phosphatidylcholine (PC)
1,2-dipalmitoyl-GPE (16:0/16:0)*	0.077	7.09E-06	4.67E-03	5.15	Lipid	Phosphatidylethanolamine
1-palmitoyl-2-linoleoyl-GPI (16:0/18:2)	0.072	3.67E-05	2.42E-02	4.44	Lipid	Phosphatidylinositol (PI)
glycocholate	0.115	4.65E-05	3.06E-02	4.33	Lipid	Primary Bile Acid Metabolism
adenine	0.086	2.98E-06	1.96E-03	5.53	Nucleotide	Purine Metabolism, Adenine containing

Note: Using longitudinal data (day 0, 3, and 7) from subjects without hemodialysis at baseline, the association between relative quantitation of each individual metabolite noted above and Creatinine (Cr) levels over time were estimated utilizing linear mixed-effects models adjusting for age, sex, baseline 25(OH)D, an absolute increase in 25(OH)D, Simplified Acute Physiology Score (SAPS) II, plasma day, admission diagnosis and individual subjects (as the random-intercept). All significant mixed-effects associations have P-value $<7.59 \times 10^{-5}$. GPG is glycerolphosphorylglycerol; GPC is glycerophosphorylcholine; GPE is glycerophosphoethanolamine; GPI is glycerophosphatidylinositol.

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SUMMARY OF MANUSCRIPT 1 AND MANUSCRIPT 2 CONCLUSIONS

This body of work illustrated two metabolomics studies in general critically ill patients from the VITdAL-ICU trial (7). Two investigations identified distinct differences in plasma metabolomics that respond to critical clinical conditions in intensive care units, inflammation and acute renal dysfunction.

In Manuscript 1, based on the longitudinal metabolomics data and mixed-effect linear regression analysis, we found 236 metabolites had significant associations with serum procalcitonin increases. The multiple metabolites were dominated in the following pathways: dicarboxylate fatty acid, branched chain amino acid, phosphatidylethanolamine, polyamine, long chain acylcarnitine, acyl choline, lysophospholipid, lysoplasmalogen, and sphingomyelin. These metabolic profile alterations indicate that inflammation is associated with energy utilization change by specific metabolic pathways in critical illness.

In Manuscript 2, using a similar method to manuscript 1, we revealed 331 metabolites were strongly associated with elevating serum creatinine in nonspecific critically ill patients. The representatives of specific metabolism pathways included acylcarnitines, branched-chain amino acids, phosphatidylethanolamines, dicarboxylate fatty acid, acyl choline, lysophospholipid class. We verified the findings were robust regardless of inflammation status. Alterations of these metabolic pathways associated with acute renal dysfunction also indicate mitochondrial dysfunction and impaired energy utilization, such as fatty acid β-oxidation.

OVERALL DISCUSSION AND PERSPECTIVES

Our metabolomics studies had several strengths. We collected a vast number of longitudinal samples at three time points to identify metabolomic profiles related to common clinical conditions in critical illness. For analyzing repeated measure data, we applied multivariable linear mixed-effects models to consider autocorrelation in subjects and confounders such as subject's characteristics (8). We adjusted the significance threshold using the false discovery rate and the Bonferroni correction for broad multiple comparisons in metabolomics data (9).

There are multiple limitations in our work. Our finding should be regarded as hypothesis generation rather than causal inference due to longitudinal cross-sectional metabolomics data. Though we applied multivariable adjustment analysis, we could not exclude residual confounding from unknown and unmeasured confounders due to nonrandomized comparisons. Furthermore, the analytic cohort from the VITdAL-ICU trial was recruited from a single large academic medical area and was a Caucasian population. This may limit the generalizability of our findings. Finally, it remains uncertain that how changes in metabolites abundance are clinically meaningful.

Metabolomics profiles in our research elucidated disturbed energy utilization and impaired mitochondrial function among critically ill patients. Identifying metabolomic patterns is a first step towards understanding energy utilization dynamics, subdivide critically ill patients into several groups, and discovering possible points of intervention in critical illness. Though further research has to verify our results, our metabolomic work provides a basis for future research into the pathophysiology and the dynamics of mitochondrial energy utilization in critical illness.

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