SEX SPECIFIC METABOLOMICS AND RESPONSE TO HIGH DOSE VITAMIN D IN CRITICAL ILLNESS: POST-HOC ANALYSES OF THE VITDAL-ICU TRIAL

By

Sowmya Chary

A Dissertation Submitted to the Faculty of Harvard Medical School in Partial Fulfillment of the Requirements for the Degree of Master of Medical Sciences in Clinical Investigation (MMSCI)

Harvard University
Boston, Massachusetts
April 30, 2020

Thesis Committee Members:
MMSCI Program Directors: Ajay K. Singh, Finnian McCausland
Primary Mentor: Kenneth B. Christopher
MMSCI Program Representative: Enid E. Martinez
External Content Advisor: Sherif Hanafy Mahmoud
Independent External Expert: Martin Ingi Sigurðsson

Area of Concentration: Metabolomics/Critical Care Outcomes

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

Primary Mentor: Kenneth B. Christopher, MD, SM

TABLE OF CONTENTS

Acknowledgements

I wish to express my deepest gratitude and appreciation to my mentor, Dr. Kenneth B. Christopher for his continued support and encouragement over the past three and a half years. He has not only taught me invaluable practical skills in study conceptualization and execution but has done so with the utmost patience and positivity. Without your guidance and insight, this work would not have been possible.

Next, I would like to thank Dr. Ajay K. Singh and Dr. Finnian McCausland for their sustained support and mentorship over the past two years. My sincerest gratitude to the rest of the thesis committee, Dr. Enid Martinez, Dr. Sherif Hanafy Mahmoud, and Dr. Martin Ingi Sigurðsson, for their encouragement and insightful feedback, and a special thank you to all who contributed to this work. I have learned immensely from you, and your feedback and advice have helped to shape and bring to fruition, this body of work.

Thank you to the program faculty, administration, and my classmates at Harvard Medical School. You have been vital in helping me grow as a clinical investigator. A special thank you to Dr. Brian Healy, for the most instrumental lectures and practical modules in understanding, designing, and interpreting observational and experimental clinical research.

Thank you to my parents, Smitha and Sri, and my sister, Sravya, for being my rock. Finally, thank you to my husband, Bhavin, for the unwavering love and support.

Overview

The National Institutes of Health issued guidance requiring equal representation of women in clinical research in 1993. However, advancements in closing the medical research gender gap are far from reaching parity. Genes related to metabolism vary between men and women and contribute to sex-specific differences in disease incidence and severity and pharmacokinetics and pharmacometabolomics of interventions. Metabolomics studies have demonstrated these differences in healthy individuals but not in the response to critical illness.

If critically ill women respond to stressors differently and plasma metabolomics differ between men and women, identifying sex-specific differences in metabolism pathways is crucial to understanding personalized medicine delivery. Furthermore, sex-specific pharmacological properties and pharmacometabolomics are poorly described in the critically ill. Studying these differences may allow for sex-specific dosage recommendations and pave the way for future studies to evaluate sex-specific differences in the response to interventions in the critically ill.

To answer these questions, we studied sex-specific plasma metabolomics differences at three time points in early critical illness and sex-specific metabolic differences in the response to high dose oral vitamin D3 in early critical illness. We hypothesized that a) significant sex-specific differences exist in plasma metabolomics of critical illness and b) significant sex-specific differences exist in the pharmacokinetic and metabolomic response to high dose oral vitamin D_3.

Title: Sex-Specific Critical Illness Metabolomics: A post-hoc analysis of the VITdAL-ICU Trial

Sowmya Chary, MBBS [1,5], Karin Amrein, MD, MSc [2], Jessica A. Lasky-Su, ScD [3], Harald Dobnig, MD [4], Kenneth B. Christopher, MD, SM [3,5]

1. Harvard Medical School, Boston, USA
2. Division of Endocrinology and Diabetology, Medical University of Graz, Graz, Austria
3. Channing Division of Network Medicine, Brigham and Women's Hospital, Boston, USA
4. Thyroid Endocrinology Osteoporosis Institute Dobnig, Graz, Austria
5. Division of Renal Medicine, Brigham and Women's Hospital, Boston, USA

Corresponding Author: Kenneth B. Christopher, MD, SM, Division of Renal Medicine, Brigham and Women's Hospital, 75 Francis Street, Boston, 02115 USA kbchristopher@bwh.harvard.edu p 617 272 0535

Author Contributions: Drs. Christopher, Amrein and Lasky-Su had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Chary, Amrein, Lasky-Su, Christopher. Acquisition, analysis, or interpretation of data: All authors. Drafting of manuscript: All authors. Statistical analysis: Amrein, Lasky-Su, Christopher. Obtained funding: Amrein, Dobnig, Christopher. Administrative technical or material support. All authors. Study Supervision: Amrein, Dobnig, Christopher.

Funding: The VITdAL-ICU trial was supported by the European Society for Clinical Nutrition and Metabolism (ESPEN), a research grant including provision of study medication from Fresenius Kabi (Germany), and the Austrian National Bank (Jubiläumsfonds, Project Nr. 14143). The metabolomics work was funded by the NIGMS/ R01 GM115774.

Running Head: Sex-specific critical illness metabolomics

Descriptor Number 4.10

Total Word Count Body 2284

This article has an online data supplement, which is accessible from this issue's table of contents online at www.atsjournals.org

Abstract

Rationale: It is unclear if the metabolic response to early critical illness differs in women and men.

Objectives: To determine the sex-specific metabolomic response early in the course of critical illness.

Methods: We performed a post-hoc metabolomics study of the VITdAL-ICU randomized, double-blind, placebo-controlled trial. Trial patients from Medical and Surgical Intensive Care Units at a tertiary university hospital with 25-hydroxyvitamin D level ≤ 20 ng/mL received either high dose oral vitamin D_3 (540,000 IU) or placebo. We performed an analysis of 578 metabolites in 1215 plasma samples from 428 subjects at randomization (day 0), day 3 and 7. Using mixed-effects modeling, we studied sex-specific changes in individual metabolites over time adjusted for age, Simplified Acute Physiology Score II, admission diagnosis, baseline 25-hydroxyvitamin D level, and 25-hydroxyvitamin D response to intervention. We employed Gaussian graphical modeling to identify groups of sex-specific metabolites that are functionally co-regulated.

Measurements and Main Results: 35% of subjects were women. In women, multiple members of the sphingomyelin and lysophospholipid metabolite classes had significantly positive Bonferroni corrected associations over time compared to men. Further, multiple representatives of the acylcarnitine, androgenic steroids, bile acid, nucleotide and amino acid metabolite classes had significantly negative Bonferroni corrected associations over time compared to men. Gaussian graphical model analyses revealed seven sex-specific functional modules at day 3 and seven at day 7.

Conclusions: Robust coordinated sex-specific metabolite differences are present early in critical illness. This work increases our understanding of the sex-specific differences in the metabolic response to critical illness.

Word Count Abstract: 249

Keywords:

Metabolite, Metabolomics, Gender, Sex, Critical Illness

Introduction

Inclusiveness of women subjects in clinical research was mandated by the National Institutes of Health in 1993. Most NIH sponsored clinical research and most other studies still do not account for sex-specific differences (1-3). Robust differences do exist between men and women with respect to disease incidence, disease severity and pharmacodynamics of interventions (4, 5). Sex-specific outcome differences are present favoring females in experimental critical illness but are not consistently observed in observational studies in critical care (6-9).

Metabolites in circulating blood are the final products of patients' cellular metabolism. A few large metabolomics studies on healthy individuals are notable for robust differences related to sex (10-12). Genes related to metabolism show variation which contribute to sexual dimorphism (11, 12). However, metabolomics on healthy subjects have little relevance to critically ill patients. Indeed the metabolic homeostasis observed in the healthy is profoundly disturbed in the critically ill (13).

Metabolomic studies performed early in critical illness can reflect illness severity and predict outcomes. But such work does not address sex-specific differences in the response to critical illness (14-16). Therefore, we studied differences between women and men with regards to changes in metabolism during critical illness. We hypothesize that significant sex-specific plasma metabolomics differences exist in the response to critical illness. We performed a post-hoc metabolomics analysis of 1215 plasma samples from 428 patients collected during the VITdAL-ICU trial (17). We assessed the effect of sex on changes in individual metabolites and plasma

metabolite families over three time points early in the course of critical illness. Further, with the metabolite change data we determined if regulated metabolite modules exist that associate with sex.

Methods

The VITdAL-ICU trial randomized 475 critically ill adult patients with 25-hydroxyvitamin D (25(OH)D) < 20 ng/mL to vitamin D_3 or placebo given orally or via nasogastric tube once at a dose of 540,000 IU followed by 90,000 IU monthly (17). Blood samples were collected on days 0 (at randomization), 3 and 7. Plasma was fractionated, aliquoted and stored at -80°C. Four hundred fifty-three trial subjects had frozen plasma available for analysis. We excluded 25 trial subjects who did not have 25(OH)D measured at day 3 following randomization.

VITdAL-ICU trial subject plasma aliquots were shipped on dry-ice to Metabolon, Inc. Following receipt, the frozen plasma samples were immediately stored at -80^{o}C. To generate metabolomic data, a total of 1215 plasma samples from 428 patients at day 0, 413 patients at day 3 and 374 patients at day 7 were analyzed using four ultra-high-performance liquid chromatography/tandem accurate mass spectrometry (UHPLC/MS/MS) methods by Metabolon, Inc. Metabolomic profiling identified 769 metabolites. We reduced baseline noise by removing metabolites with the lowest interquartile range of variability, leaving 578 metabolites. Metabolomic data underwent cube root transformation and Pareto scaling to generate a "normal" distribution.

At randomization (day 0) data was 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). The quality of the multivariate model developed was described by R2 and Q2 corresponding to goodness of fit and predictive performance, respectively. Permutation testing was performed to validate the OPLS-DA model (18, 19). Sevenfold cross-validation analysis of variance (CV-ANOVA) was utilized to determine OPLS-DA model significance (19).

For single time point data, correlations between individual metabolites and sex at day 0, 3 or 7 were separately determined utilizing linear regression models correcting for age, Simplified Acute Physiology Score (SAPS) II, admission diagnosis and 25(OH)D at day 0. Additionally, for day 3 and 7, the linear regression models were corrected for absolute change in 25(OH)D level at day 3. A multiple test-corrected threshold of $P < 8.65 \times 10^{-5}$ was used to identify all significant associations in the single time point data (20). We employed rain plots (21) to visualize effect size, significance, clustering and trends across days 0, 3 and 7. Rain plots were produced based on hierarchical clustering in R-3.6.2 adapted from source code published by Henglin et.al. (21).

For repeated measures data, correlations between individual metabolites and sex over time (day 0, 3 and 7) were determined utilizing linear mixed models correcting for age, SAPS II, admission diagnosis, 25(OH)D at day 0, absolute change in 25(OH)D level at day 3 and plasma day (as the random-intercept). For data visualization purposes, a bipartite graph (22) was generated of metabolites which were significantly changed (increased or decreased) with sex. In women

subjects, mixed effects logistic regression was used to estimate the odds of 28-day mortality of individual metabolites adjusted for age, SAPS II, admission diagnosis, 25(OH)D at day 0, absolute change in 25(OH)D level at day 3 and plasma day (as the random-intercept). A multiple test-corrected threshold of $P < 8.65 \times 10^{-5}$ was used to identify all significant associations in the dynamic data (20). All mixed models were analyzed using STATA 14.1MP (College Station, TX).

As inflammation is important in response to critical illness, we evaluated a potential mediating effect of procalcitonin or c-reactive protein on the association between sex and individual metabolite abundance adjusted for age, SAPS II, admission diagnosis, 25(OH)D at day 0 and absolute change in 25(OH)D level at day 3. Analyses were performed on each of the 578 metabolites at day 3 using the R package mediation (23) to obtain bootstrap P values (N = 2000 samples) for the mediation effect of procalcitonin or c-reactive protein. Significant mediation was present if the p value was<0.01 and 10% or more of the association was mediated through procalcitonin or c-reactive protein levels (24, 25).

To identify sex-specific modules from metabolomics data, we estimated Gaussian graphical models (GGMs) for day 3 and 7. Modules serve to reconstruct pathway reactions from metabolomics data. GGMs are determined utilizing partial pairwise Pearson correlation coefficients following the removal of the effects of all other metabolites and covariates (26). GGMs are representations of the linear association between two metabolites corrected for other confounding variables in multivariate Gaussian distributions. We inferred a sex-specific network for relative metabolite abundance. We included age, Simplified Acute Physiology Score (SAPS) II,

admission diagnosis, 25(OH)D at day 0, absolute change in 25(OH)D level at day 3 and plasma day as covariates into the model. Edges between metabolites were allotted if both their Pearson correlations and partial correlations remained statistically significant at $P < 0.05$ following Bonferroni correction for 578 metabolites (27). GGMs were produced using the GeneNet R package, version 1.2.13 in R-3.6.2 adapted from source code published by Do et.al. (27).

Results

Baseline characteristics of the analytic cohort were balanced between subjects stratified by sex for C-reactive protein, SAPS II, baseline 25(OH)D levels, intervention status and ICU type. Differences existed by sex with respect to age (Table 1 and Supplemental Table 1). The overall 28-day mortality of the 428 subject analytic cohort was 22.2%. The 28-day mortality in women was 22.5% and in men was 22.0%.

In day 0 plasma samples (N=432), significant differences exist in 12 individual metabolites (all multiple test-corrected threshold of $P < 8.65 \times 10^{-5}$) and in metabolomic profiles (CV-ANOVA p value<0.001) in female subjects relative to males (Supplemental Table 3). Day 0 differences are present with increased sphingomyelins and decreased androgenic steroids in women relative to men (Supplemental Table 4).

In linear regression models of metabolite data from single time points (day 0, 3 or 7), we find significant differences exist in 51 individual metabolites at 1 or more time point (all multiple test-corrected threshold of $P < 8.65 \times 10^{-5}$, $-\log_{10}(p) > 4.06$). The rain plots show the separation of

metabolites that are increased (Figure 1) or decreased (Figure 2) in women relative to men, with greater significance shown by increased size of the circles. In the single time point data, women primarily have significant increases in sphingomyelins and lysophopholipids relative to men. Decreases in androgenic steroids as well as bile acid and amino acid metabolism are found in women relative to men.

In mixed effects modeling of 1215 total day 0, 3 and 7 plasma samples from the analytic cohort (N=432), 50 metabolites had significantly positive associations in women relative to men dominated by increases in sphingomyelins and lysophospholipids *(*Summarized data in Table 2, Full data in Supplemental Table 5, Figure 3). 105 metabolites had significantly negative associations in women relative to men primarily by decreases in acylcarnitine, androgenic steroid, bile acid, nucleotide and amino acid metabolites (Summarized data in Table 3, Full data in Supplemental Table 6, Figure 3).

We next explored the sex-specific associations of individual metabolites and 28-day mortality. We compared mixed effects modeling of 441 total day 0, 3 and 7 plasma samples from women in the analytic cohort (N=151) to mixed effects modeling of 814 total day 0, 3 and 7 plasma samples from men in the analytic cohort (N=277). The data show that elevations in short chain acylcarnitines C4-C8 and branched-chain amino acids are significantly associated with three-fold increases in 28-day mortality in women but not men (Supplemental Table 7).

We next investigated sex-specific metabolic networks by measuring pairwise correlations in metabolites which have similar effects via Gaussian graphical models (GGMs). The GGMs analyses revealed seven sex-specific functional modules at day 3 and seven at day 7 (Supplementary tables 8 & 9). Similar to the mixed effects analyses, metabolism of branched chain amino acids, bile acids, androgenic steroids and lysophospholipids are prominently featured in the sex specific GGM modules. Metabolites within in each functional module were either increased or decreased in women in unison and had biological or functional similarity. Individual metabolites which are not associated with sex in our mixed effects analysis also do contribute to significant sex-specific modules (Supplementary tables 8 & 9: Modules B, E, H, I, K, and M).

We finally focused on the potential mediation of the relationship between individual metabolite abundance and sex by inflammation status. Mediation analyses in day 3 data revealed no influence of Procalcitonin or of C-reactive protein on the associations between sex and each of the individual 578 metabolites (all p values were > 0.01 using 2000 bootstrap samples).

Discussion

Our large post-hoc metabolomics study identified metabolites early in critical illness with sex-specific associations. In our single time point data and our mixed methods analysis, we consistently find robust increases and decreases in groups of metabolites along similar sub-pathways that have conserved function. Further, we illustrate how groups of metabolites with similar sex-specific effects form modules which highlight the same sub-pathways as our single

time point data and our mixed methods analysis (12). These modules serve to focus potential biological interpretation of our sex-specific metabolomics observations (28). All three analyses highlight the importance of sex-specific metabolism in critical illness related to branched chain amino acids, bile acids, androgenic steroids and lysophospholipids.

Metabolism and homeostasis differ by sex (29, 30). Critically ill patients preferentially catabolize fatty acids and amino acids for the mitochondrial production of energy. Female mitochondria have higher oxidative capacity, produce less reactive oxygen species, and preferentially utilize lipids for bioenergetics (31-33). Elevated circulating even-chain C4 – C22 acylcarnitines are due to incomplete mitochondrial β-oxidation of fatty acids (34, 35). In healthy adults, circulating acylcarnitines are generally less abundant in women compared to men (12). Increases in circulating acylcarnitines associate with adverse outcomes in critical illness (15). We demonstrate that 15 circulating acylcarnitines are significantly lower in women early in critical illness (Supplemental Table 6). Our observed decreased even-chain acylcarnitines in women relative to men probably reflects more efficient fatty acid β-oxidation indicative of sex-specific differences in mitochondrial response to critical illness.

The circulating amino acid pool is supplied by dietary amino acids, endogenous amino acid synthesis and cellular protein turnover (36). Increases in circulating amino acids during critical illness are due to protein catabolism (37). Skeletal muscle protein is rapidly metabolized in response to severity of illness to provide substrate for liver gluconeogenesis, immune function support and immunoglobulin synthesis (38). Further, amino acid catabolism is a source for

circulating C3, C4 and C5 acylcarnitines (35). Our findings of decreases in C3, C4 and C5 acylcarnitines as well as in multiple amino acid metabolite sub-pathways may reflect sex-specific protein catabolism and energy substrate utilization during critical illness.

We observe substantial sex-specific differences in lipid metabolism. Our novel observations suggest such differences specifically exist in sphingomyelins, plasmalogens and lysophospholipids, which are mediators of the stress response, antioxidants and immunomodulation, respectively. Sex-specific differences in lipid profiles are due to sex chromosome and sex-specific hormone action including estrogen, progesterone and androgens (39). Sex-specific differences exist in the utilization of carbohydrates and lipids as energy. At homeostasis women incorporate free fatty acids into triglycerides whereas men oxidize circulating free fatty acids (40). With increased energy needs during exercise, women oxidize lipids in favor of carbohydrates while men utilize carbohydrates (41). Further, women have less free fatty acid-induced insulin resistance compared to men (42). Healthy adult women have increased circulating sphingomyelins compared to men (43, 44).

Though sex-specific differences in bile acid synthesis are frequently noted (45) such differences in bile acid homeostasis are not well characterized (46). The cytochrome P450s are prominent enzymes important for bile synthesis (45) and are regulated in a sex-specific manner (47, 48). Bile acids are known to activate the nuclear receptors farnesoid X receptor, pregnane X receptor and vitamin D receptor as well as the G-protein-coupled receptor TGR5. Such bile acid receptor activation results in gene expression which alters metabolism of bile acids, glucose, lipids, energy

and inflammation (49). As elevation in blood bile acids are common in critical illness (50), and the synthesis and pool composition of bile acids are sex-specific, such differences have widespread downstream metabolism pathway effects.

Our novel study approach has several strengths. The use of a large number of plasma samples at multiple time points early in critical illness allows for a dynamic view into sex-specific metabolomics. Linear mixed models are extremely useful for metabolomic data measured at multiple time points as they remove confounding variables with a fixed-effect (age, SAPS II, etc.) and also those with a random-effect (sampling day) (51, 52). Linear mixed models account for the longitudinal nature of our data and can model the trajectories for individual metabolites abundance over time. Further, our use of clinical trial data allows for modelling and normalization of metabolite abundance via adjustment for subject characteristics (53). To account for multiple comparisons we utilized a conservative Bonferroni corrected P value $< 8.65 \times 10^{-5}$ (20). Finally, we observe differences in metabolism known to be associated with sex, thus increasing the biological plausibility and relevance of our work.

We do acknowledge potential limitations to our approach. Despite multivariable adjustment, our approach is subject to bias. Though our samples are derived from a randomized controlled trial, our study design is observational thus causal inference may be limited. VITdAL-ICU trial subjects were all Caucasian with 25(OH)D< 20 ng/ml, thus may not be representative of all critically ill. Furthermore, our study is an exploratory analysis requiring subsequent confirmation.

Conclusion

In a large metabolomics study of a trial biorepository, we demonstrate substantial differences between women and men in the plasma metabolomics of early critical illness. We find that women respond to critical illness stressors in a dramatically different fashion than men. Studying such differences in metabolism pathways over time is an essential step in understanding how to provide personalized medicine in the critically ill.

Figure Legends

Figure 1. Rain Plot of single time point metabolites increased in women. Correlations between individual metabolites and sex at day 0, 3 or 7 were determined utilizing linear regression models correcting for age, Simplified Acute Physiology Score (SAPS) II, admission diagnosis and 25(OH)D at day 0. Day 3 and 7 estimates were also corrected 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 women compared to men. Statistical significance is the multiple test-corrected threshold of $-\log_{10}(p) > 4.06$ which is equivalent to $P < 8.65 \times 10^{-5}$.

Figure 2. Rain Plot of single time point metabolites decreased in women. Correlations between individual metabolites and sex at day 0, 3 or 7 were determined utilizing linear regression models correcting for age, Simplified Acute Physiology Score (SAPS) II, admission diagnosis and 25(OH)D at day 0. Day 3 and 7 estimates were also corrected 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 blue fill color represents a decrease in effect size for that metabolite in women compared to men. Statistical significance is the multiple test-corrected threshold of $-\log_{10}(p) > 4.06$ which is equivalent to $P < 8.65 \times 10^{-5}$.

Figure 3. Circos Plot of metabolites over time. Bipartite graph of metabolites measured in 1215 plasma samples from 428 patients. Metabolites shown are determined to be significantly increased or decreased in women relative to men over the first seven days following trial enrollment. Graph connects the increase or decrease in metabolite on the left side with individual metabolites on the right side. Width of curves indicates strength of the significance ($-\log_{10}$(p) value) as determined by mixed-effects regression. Colors differ for each sub-pathway (i.e. amino acid metabolites are red). All curves shown have P value $< 8.65 \times 10^{-5}$.

Acknowledgements

This article is dedicated to the memory of our dear friend and colleague Nathan Edward Hellman, MD, PhD.

Conflict of Interest: Dr. Lasky-Su served as a consultant and received personal fees from Metabolon, Inc., outside the submitted work. Dr. Amrein reports receiving lecture fees from Fresenius Kabi. Dr. Dobnig reports receiving lecture fees from Fresenius Kabi. No other financial or other relationships exist that might lead to a conflict of interest.

References

1. Geller SE, Koch AR, Roesch P, Filut A, Hallgren E, Carnes M. The More Things Change, the More They Stay the Same: A Study to Evaluate Compliance With Inclusion and Assessment of Women and Minorities in Randomized Controlled Trials. Academic medicine : journal of the Association of American Medical Colleges. 2018;93(4):630-5.
2. Woitowich NC, Woodruff TK. Opinion: Research community needs to better appreciate the value of sex-based research. Proc Natl Acad Sci U S A. 2019;116(15):7154-6.
3. Kim AM, Tingen CM, Woodruff TK. Sex bias in trials and treatment must end. Nature. 2010;465(7299):688-9.
4. Regitz-Zagrosek V. Sex and gender differences in health. Science & Society Series on Sex and Science. EMBO Rep. 2012;13(7):596-603.
5. Gandhi M, Aweeka F, Greenblatt RM, Blaschke TF. Sex differences in pharmacokinetics and pharmacodynamics. Annu Rev Pharmacol Toxicol. 2004;44:499-523.
6. van Eijk LT, Dorresteijn MJ, Smits P, van der Hoeven JG, Netea MG, Pickkers P. Gender differences in the innate immune response and vascular reactivity following the administration of endotoxin to human volunteers. Crit Care Med. 2007;35(6):1464-9.
7. Sheth SU, Palange D, Xu DZ, Wei D, Feketeova E, Lu Q, et al. Testosterone depletion or blockade in male rats protects against trauma hemorrhagic shock-induced distant organ injury by limiting gut injury and subsequent production of biologically active mesenteric lymph. J Trauma. 2011;71(6):1652-8.
8. Samuelsson C, Sjoberg F, Karlstrom G, Nolin T, Walther SM. Gender differences in outcome and use of resources do exist in Swedish intensive care, but to no advantage for women of premenopausal age. Crit Care. 2015;19:129.
9. Mahmood K, Eldeirawi K, Wahidi MM. Association of gender with outcomes in critically ill patients. Crit Care. 2012;16(3):R92.
10. Dunn WB, Lin W, Broadhurst D, Begley P, Brown M, Zelena E, et al. Molecular phenotyping of a UK population: defining the human serum metabolome. Metabolomics. 2015;11:9-26.
11. Kolz M, Johnson T, Sanna S, Teumer A, Vitart V, Perola M, et al. Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. PLoS Genet. 2009;5(6):e1000504.
12. Mittelstrass K, Ried JS, Yu Z, Krumsiek J, Gieger C, Prehn C, et al. Discovery of sexual dimorphisms in metabolic and genetic biomarkers. PLoS Genet. 2011;7(8):e1002215.
13. Kiehntopf M, Nin N, Bauer M. Metabolism, metabolome, and metabolomics in intensive care: is it time to move beyond monitoring of glucose and lactate? Am J Respir Crit Care Med. 2013;187(9):906-7.
14. Langley RJ, Tipper JL, Bruse S, Baron RM, Tsalik EL, Huntley J, et al. Integrative "omic" analysis of experimental bacteremia identifies a metabolic signature that distinguishes human sepsis from systemic inflammatory response syndromes. Am J Respir Crit Care Med. 2014;190(4):445-55.
15. Langley RJ, Tsalik EL, van Velkinburgh JC, Glickman SW, Rice BJ, Wang C, et al. An integrated clinico-metabolomic model improves prediction of death in sepsis. Sci Transl Med. 2013;5(195):195ra95.

16. Johansson PI, Nakahira K, Rogers AJ, McGeachie MJ, Baron RM, Fredenburgh LE, et al. Plasma mitochondrial DNA and metabolomic alterations in severe critical illness. Crit Care. 2018;22(1):360.
17. Amrein K, Schnedl C, Holl A, Riedl R, Christopher KB, Pachler C, et al. Effect of high-dose vitamin D3 on hospital length of stay in critically ill patients with vitamin D deficiency: the VITdAL-ICU randomized clinical trial. JAMA. 2014;312(15):1520-30.
18. Westerhuis JA, Hoefsloot HCJ, Smit S, Vis DJ, Smilde AK, van Velzen EJJ, et al. Assessment of PLSDA cross validation. Metabolomics 2008;4(1):81–9.
19. Eriksson L, Trygg J, Wold S. CV-ANOVA for significance testing of PLS and OPLS models. Journal of Chemometrics. 2008;22(11-12):594-600.
20. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A. 2003;100(16):9440-5.
21. Henglin M, Niiranen T, Watrous JD, Lagerborg KA, Antonelli J, Claggett BL, et al. A Single Visualization Technique for Displaying Multiple Metabolite-Phenotype Associations. Metabolites. 2019;9(7).
22. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, et al. Circos: an information aesthetic for comparative genomics. Genome Res. 2009;19(9):1639-45.
23. Dustin T, Yamamoto T, Hirose K, Keele L, Imai K. mediation: R package for causal mediation analysis. Journal of Statistical Software. 2014;59(5):1-38.
24. Masuch A, Pietzner M, Bahls M, Budde K, Kastenmuller G, Zylla S, et al. Metabolomic profiling implicates adiponectin as mediator of a favorable lipoprotein profile associated with NT-proBNP. Cardiovasc Diabetol. 2018;17(1):120.
25. Pietzner M, Budde K, Homuth G, Kastenmuller G, Henning AK, Artati A, et al. Hepatic Steatosis Is Associated With Adverse Molecular Signatures in Subjects Without Diabetes. J Clin Endocrinol Metab. 2018;103(10):3856-68.
26. Krumsiek J, Suhre K, Illig T, Adamski J, Theis FJ. Gaussian graphical modeling reconstructs pathway reactions from high-throughput metabolomics data. BMC Syst Biol. 2011;5:21.
27. Do KT, Pietzner M, Rasp DJ, Friedrich N, Nauck M, Kocher T, et al. Phenotype-driven identification of modules in a hierarchical map of multifluid metabolic correlations. NPJ Syst Biol Appl. 2017;3:28.
28. Mitra K, Carvunis AR, Ramesh SK, Ideker T. Integrative approaches for finding modular structure in biological networks. Nature reviews Genetics. 2013;14(10):719-32.
29. Murphy MO, Loria AS. Sex-specific effects of stress on metabolic and cardiovascular disease: are women at higher risk? Am J Physiol Regul Integr Comp Physiol. 2017;313(1):R1-R9.
30. Sugiyama MG, Agellon LB. Sex differences in lipid metabolism and metabolic disease risk. Biochem Cell Biol. 2012;90(2):124-41.
31. Ventura-Clapier R, Moulin M, Piquereau J, Lemaire C, Mericskay M, Veksler V, et al. Mitochondria: a central target for sex differences in pathologies. Clin Sci (Lond). 2017;131(9):803-22.
32. Vina J, Borras C. Women live longer than men: understanding molecular mechanisms offers opportunities to intervene by using estrogenic compounds. Antioxid Redox Signal. 2010;13(3):269-78.
33. Austad SN, Fischer KE. Sex Differences in Lifespan. Cell Metab. 2016;23(6):1022-33.

34. Van Hove JL, Zhang W, Kahler SG, Roe CR, Chen YT, Terada N, et al. Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency: diagnosis by acylcarnitine analysis in blood. Am J Hum Genet. 1993;52(5):958-66.
35. Koves TR, Ussher JR, Noland RC, Slentz D, Mosedale M, Ilkayeva O, et al. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. Cell Metab. 2008;7(1):45-56.
36. Ochoa Gautier JB, Martindale RG, Rugeles SJ, Hurt RT, Taylor B, Heyland DK, et al. How Much and What Type of Protein Should a Critically Ill Patient Receive? Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition. 2017;32(1_suppl):6S-14S.
37. Preiser JC, Ichai C, Orban JC, Groeneveld AB. Metabolic response to the stress of critical illness. Br J Anaesth. 2014;113(6):945-54.
38. Martindale RG, Heyland DK, Rugeles SJ, Wernerman J, Weijs PJ, Patel JJ, et al. Protein Kinetics and Metabolic Effects Related to Disease States in the Intensive Care Unit. Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition. 2017;32(1_suppl):21S-9S.
39. Varlamov O, Bethea CL, Roberts CT, Jr. Sex-specific differences in lipid and glucose metabolism. Front Endocrinol (Lausanne). 2014;5:241.
40. Uranga AP, Levine J, Jensen M. Isotope tracer measures of meal fatty acid metabolism: reproducibility and effects of the menstrual cycle. Am J Physiol Endocrinol Metab. 2005;288(3):E547-55.
41. Henderson GC. Sexual dimorphism in the effects of exercise on metabolism of lipids to support resting metabolism. Front Endocrinol (Lausanne). 2014;5:162.
42. Frias JP, Macaraeg GB, Ofrecio J, Yu JG, Olefsky JM, Kruszynska YT. Decreased susceptibility to fatty acid-induced peripheral tissue insulin resistance in women. Diabetes. 2001;50(6):1344-50.
43. Weir JM, Wong G, Barlow CK, Greeve MA, Kowalczyk A, Almasy L, et al. Plasma lipid profiling in a large population-based cohort. J Lipid Res. 2013;54(10):2898-908.
44. Trabado S, Al-Salameh A, Croixmarie V, Masson P, Corruble E, Feve B, et al. The human plasma-metabolome: Reference values in 800 French healthy volunteers; impact of cholesterol, gender and age. PLoS One. 2017;12(3):e0173615.
45. Phelps T, Snyder E, Rodriguez E, Child H, Harvey P. The influence of biological sex and sex hormones on bile acid synthesis and cholesterol homeostasis. Biol Sex Differ. 2019;10(1):52.
46. Baars A, Oosting A, Lohuis M, Koehorst M, El Aidy S, Hugenholtz F, et al. Sex differences in lipid metabolism are affected by presence of the gut microbiota. Sci Rep. 2018;8(1):13426.
47. Penaloza CG, Estevez B, Han DM, Norouzi M, Lockshin RA, Zakeri Z. Sex-dependent regulation of cytochrome P450 family members Cyp1a1, Cyp2e1, and Cyp7b1 by methylation of DNA. FASEB J. 2014;28(2):966-77.
48. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocrine reviews. 2011;32(1):81-151.
49. de Aguiar Vallim TQ, Tarling EJ, Edwards PA. Pleiotropic roles of bile acids in metabolism. Cell Metab. 2013;17(5):657-69.

50. Vanwijngaerden YM, Wauters J, Langouche L, Vander Perre S, Liddle C, Coulter S, et al. Critical illness evokes elevated circulating bile acids related to altered hepatic transporter and nuclear receptor expression. Hepatology. 2011;54(5):1741-52.
51. Mei Y, Kim BS, Tsui K. Linear mixed effects models for feature selection in high dimensional NMR spectra. Exprt Syst Applic. 2009;36:4703–8.
52. Ernest B, Gooding JR, Campagna SR, Saxton AM, Voy BH. MetabR: an R script for linear model analysis of quantitative metabolomic data. BMC Res Notes. 2012;5:596.
53. Wanichthanarak K, Jeamsripong S, Pornputtapong N, Khoomrung S. Accounting for biological variation with linear mixed-effects modelling improves the quality of clinical metabolomics data. Comput Struct Biotechnol J. 2019;17:611-8.

Table 1. Cohort Characteristics

Characteristic	Female	Male	Total	P-value
No.	151	277	428	
Age years Mean (SD)	68.2 (13.3)	62.0 (15.3)	64.2 (14.9)	<0.001*
Day 0 25(OH)D ng/ml Mean (SD)	13.2 (5.7)	14.4 (10.1)	13.9 (8.8)	0.17*
SAPS II Mean (SD)	34.6 (14.7)	32.7 (15.8)	33.4 (15.4)	0.24*
Day 0 C-reactive protein µg/mL Mean (SD)	119.9 (96.4)	127.6 (86.0)	124.9 (89.8)	0.40*
Day 0 Procalcitonin ng/ml Median [IQR]	0.45 [0.14, 1.98]	0.77 [0.20, 3.02]	0.66 [0.17, 2.79]	<0.001†
Vitamin D_3 Intervention No. (%)	78 (51.7)	134 (48.4)	212 (49.5)	0.52
Change in 25(OH)D ng/ml Mean (SD)	11.3 (18.0)	10.0 (15.5)	10.4 (16.4)	0.43*
ICU				0.22
Anesthesia ICU No. (%)	24 (15.9)	59 (21.3)	83 (19.4)	
Cardiac Surgery ICU No. (%)	42 (27.8)	84 (30.3)	126 (29.4)	
Surgical ICU No. (%)	7 (4.6)	16 (5.8)	23 (5.4)	
Medicine ICU No. (%)	31 (20.5)	59 (21.3)	90 (21.0)	
Neurological ICU No. (%)	47 (31.1)	59 (21.3)	106 (24.8)	

Note: Data presented as n (%) unless otherwise indicated. P values determined by chi-square unless designated by (*) then P value determined by ANOVA or by (†) determined by Kruskal-Wallis test.

Table 2. Metabolites significantly increased in Women relative to Men over time

Metabolite	P-value	Bonferroni corrected P-value	-log10p	β Coefficient	Super Pathway	Sub Pathway
1-linoleoyl-GPE (18:2)	3.71 E-15	2.14 E-12	14.43	0.21	Lipid	Lysophospholipid
1-palmitoyl-GPA (16:0)	1.77 E-11	1.02 E-08	10.75	0.20	Lipid	Lysophospholipid
1-linoleoyl-GPA (18:2)	5.69 E-10	3.29 E-07	9.24	0.20	Lipid	Lysophospholipid
1-linolenoyl-GPC (18:3)	1.57 E-11	9.08 E-09	10.80	0.20	Lipid	Lysophospholipid
1-stearoyl-GPE (18:0)	6.74 E-16	3.90 E-13	15.17	0.19	Lipid	Lysophospholipid
1-palmitoleoyl-GPC (16:1)	5.95 E-11	3.44 E-08	10.23	0.17	Lipid	Lysophospholipid
1-arachidonoyl-GPE (20:4n6)	2.74 E-10	1.58 E-07	9.56	0.16	Lipid	Lysophospholipid
2-stearoyl-GPE (18:0)	2.13 E-09	1.23 E-06	8.67	0.16	Lipid	Lysophospholipid
1-oleoyl-GPE (18:1)	5.81 E-10	3.36 E-07	9.24	0.16	Lipid	Lysophospholipid
2-palmitoyl-GPC (16:0)	7.59 E-09	4.39 E-06	8.12	0.15	Lipid	Lysophospholipid
1-lignoceroyl-GPC (24:0)	3.89 E-06	2.25 E-03	5.41	0.15	Lipid	Lysophospholipid
1-linoleoyl-GPI (18:2)	9.30 E-08	5.37 E-05	7.03	0.14	Lipid	Lysophospholipid
1-arachidonoyl-GPC (20:4)	8.26 E-07	4.78 E-04	6.08	0.13	Lipid	Lysophospholipid
Sphingomyelin (d18:2/14:0, d18:1/14:1)	6.93 E-29	4.01 E-26	28.16	0.28	Lipid	Sphingomyelins
Sphingomyelin (d17:2/16:0, d18:2/15:0)	1.33 E-21	7.70 E-19	20.88	0.26	Lipid	Sphingomyelins
Sphingomyelin (d17:1/14:0, d16:1/15:0)	1.92 E-18	1.11 E-15	17.72	0.24	Lipid	Sphingomyelins
Sphingomyelin (d18:2/21:0, d16:2/23:0)	4.11 E-13	2.38 E-10	12.39	0.18	Lipid	Sphingomyelins
Sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)	3.76 E-10	2.17 E-07	9.43	0.17	Lipid	Sphingomyelins
Sphingomyelin (d18:1/19:0, d19:1/18:0)	1.28 E-09	7.38 E-07	8.89	0.15	Lipid	Sphingomyelins
Sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)	2.74 E-09	1.58 E-06	8.56	0.15	Lipid	Sphingomyelins
Tricosanoyl sphingomyelin (d18:1/23:0)	5.28 E-08	3.05 E-05	7.28	0.13	Lipid	Sphingomyelins
Sphingomyelin (d18:1/25:0, d19:0/24:1, d20:1/23:0, d19:1/24:0)	4.60 E-05	2.66 E-02	4.34	0.11	Lipid	Sphingomyelins

Note: Significant results presented following mixed effects modeling of each of the 578 individual metabolites measured at day 0, 3 and 7. All estimates adjusted for age, Simplified Acute Physiology Score (SAPS) II, admission diagnosis, 25(OH)D at day 0, absolute change in 25(OH)D level at day 3 and plasma day (as the random-intercept). A multiple test-corrected threshold of $P < 8.65 \times 10^{-5}$ was used to identify all significant associations. GPC is glycerophosphorylcholine; GPE is glycerophosphoethanolamine; GPI is glycosylphosphatidylinositol.

Table 3. Metabolites significantly decreased in Women relative to Men over time

Metabolite	P-value value	Bonferroni corrected P-value	-log10p	β Coefficient	Super Pathway	Sub Pathway
N-acetylvaline	2.55 E-05	1.47 E-02	4.59	-0.10	Amino Acid	Leucine, Isoleucine and Valine Metabolism
N-acetylleucine	7.65 E-06	4.42 E-03	5.12	-0.12	Amino Acid	Leucine, Isoleucine and Valine Metabolism
Alpha-hydroxyisocaproate	9.27 E-07	5.36 E-04	6.03	-0.13	Amino Acid	Leucine, Isoleucine and Valine Metabolism
3-hydroxyisobutyrate	1.37 E-06	7.90 E-04	5.86	-0.13	Amino Acid	Leucine, Isoleucine and Valine Metabolism
3-hydroxy-2-ethylpropionate	2.06 E-09	1.19 E-06	8.69	-0.15	Amino Acid	Leucine, Isoleucine and Valine Metabolism
2-hydroxy-3-methylvalerate	5.00 E-08	2.89 E-05	7.30	-0.16	Amino Acid	Leucine, Isoleucine and Valine Metabolism
2,3-dihydroxy-2-methylbutyrate	4.77 E-07	2.76 E-04	6.32	-0.17	Amino Acid	Leucine, Isoleucine and Valine Metabolism
Beta-hydroxyisovalerate	4.04 E-12	2.33 E-09	11.39	-0.19	Amino Acid	Leucine, Isoleucine and Valine Metabolism
Glycochenodeoxycholate	1.59 E-06	9.18 E-04	5.80	-0.20	Lipid	Primary Bile Acid Metabolism
Taurocholate	4.19 E-05	2.42 E-02	4.38	-0.21	Lipid	Primary Bile Acid Metabolism
Glycochenodeoxycholate glucuronide	2.94 E-12	1.70 E-09	11.53	-0.28	Lipid	Primary Bile Acid Metabolism
Taurochenodeoxycholate	2.20 E-08	1.27 E-05	7.66	-0.28	Lipid	Primary Bile Acid Metabolism
Glycochenodeoxycholate sulfate	4.26 E-11	2.46 E-08	10.37	-0.32	Lipid	Primary Bile Acid Metabolism
Glycodeoxycholate sulfate	4.01 E-06	2.32 E-03	5.40	-0.23	Lipid	Secondary Bile Acid Metabolism
Glycolithocholate sulfate	5.82 E-09	3.36 E-06	8.24	-0.27	Lipid	Secondary Bile Acid Metabolism
Glycocholenate sulfate	6.22 E-14	3.59 E-11	13.21	-0.28	Lipid	Secondary Bile Acid Metabolism
Taurolithocholate 3-sulfate	3.11 E-12	1.80 E-09	11.51	-0.34	Lipid	Secondary Bile Acid Metabolism
Taurocholenate sulfate	1.03 E-16	5.94 E-14	15.99	-0.38	Lipid	Secondary Bile Acid Metabolism

Note: Significant results presented following mixed effects modeling of each of the 578 individual metabolites measured at day 0, 3 and 7. All estimates adjusted for age, Simplified Acute Physiology Score (SAPS) II, admission diagnosis, 25(OH)D at day 0, absolute change in 25(OH)D level at day 3 and plasma day (as the random-intercept). A multiple test-corrected threshold of $P < 8.65 \times 10^{-5}$ was used to identify all significant associations.

Figure 1.

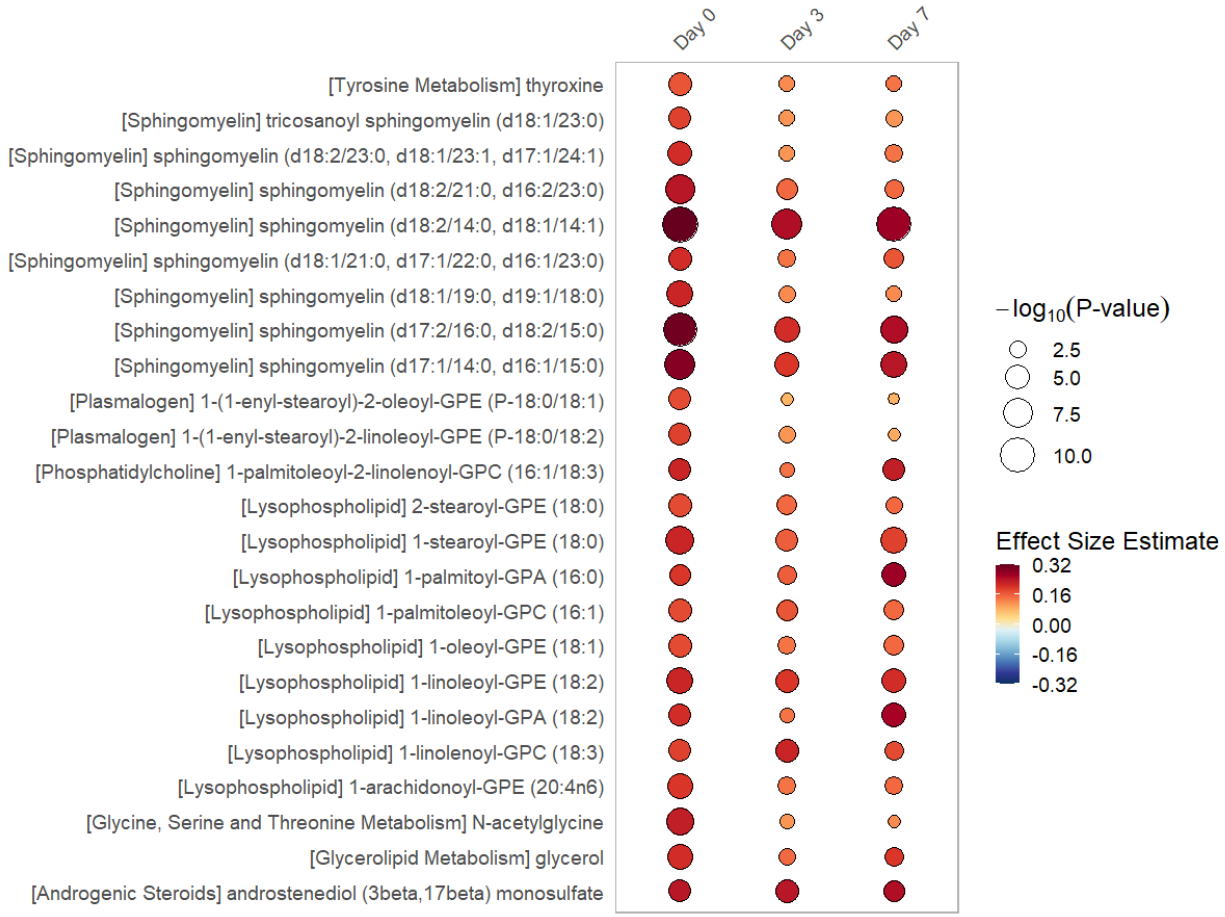

Day 0
Day 3
Day 7
[Tyrosine Metabolism] thyroxine
[Sphingomyelin] tricosanoyl sphingomyelin (d18:1/23:0)
[Sphingomyelin] sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)
[Sphingomyelin] sphingomyelin (d18:2/21:0, d16:2/23:0)
[Sphingomyelin] sphingomyelin (d18:2/14:0, d18:1/14:1)
[Sphingomyelin] sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)
[Sphingomyelin] sphingomyelin (d18:1/19:0, d19:1/18:0)
[Sphingomyelin] sphingomyelin (d17:2/16:0, d18:2/15:0)
[Sphingomyelin] sphingomyelin (d17:1/14:0, d16:1/15:0)
[Plasmalogen] 1-(1-enyl-stearoyl)-2-oleoyl-GPE (P-18:0/18:1)
[Plasmalogen] 1-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2)
[Phosphatidylcholine] 1-palmitoleoyl-2-linolenoyl-GPC (16:1/18:3)
[Lysophospholipid] 2-stearoyl-GPE (18:0)
[Lysophospholipid] 1-stearoyl-GPE (18:0)
[Lysophospholipid] 1-palmitoyl-GPA (16:0)
[Lysophospholipid] 1-palmitoleoyl-GPC (16:1)
[Lysophospholipid] 1-oleoyl-GPE (18:1)
[Lysophospholipid] 1-linoleoyl-GPE (18:2)
[Lysophospholipid] 1-linoleoyl-GPA (18:2)
[Lysophospholipid] 1-linolenoyl-GPC (18:3)
[Lysophospholipid] 1-arachidonoyl-GPE (20:4n6)
[Glycine, Serine and Threonine Metabolism] N-acetylglycine
[Glycerolipid Metabolism] glycerol
[Androgenic Steroids] androstenediol (3beta,17beta) monosulfate
−log10(P-value)
2.5
5.0
7.5
10.0
Effect Size Estimate
0.32
0.16
0.00
-0.16
-0.32

Figure 2.

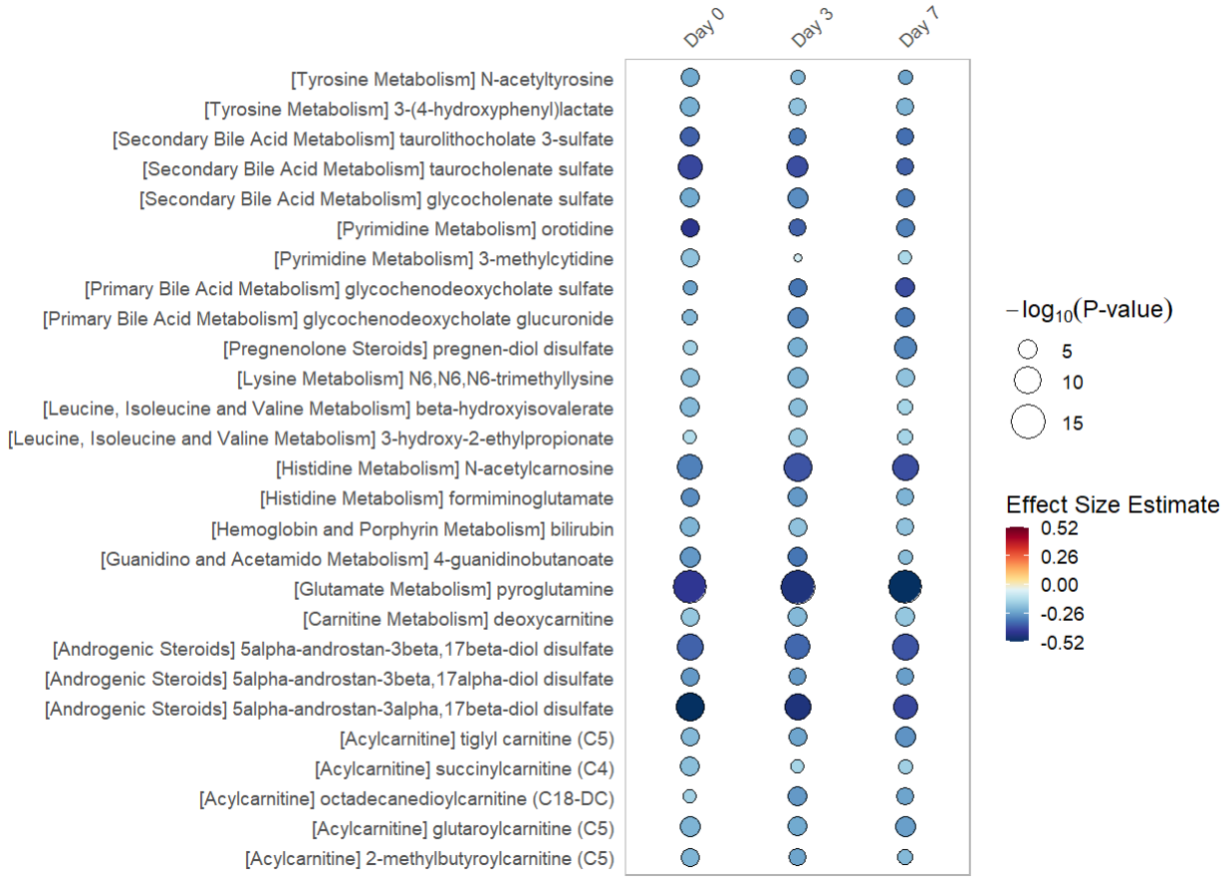
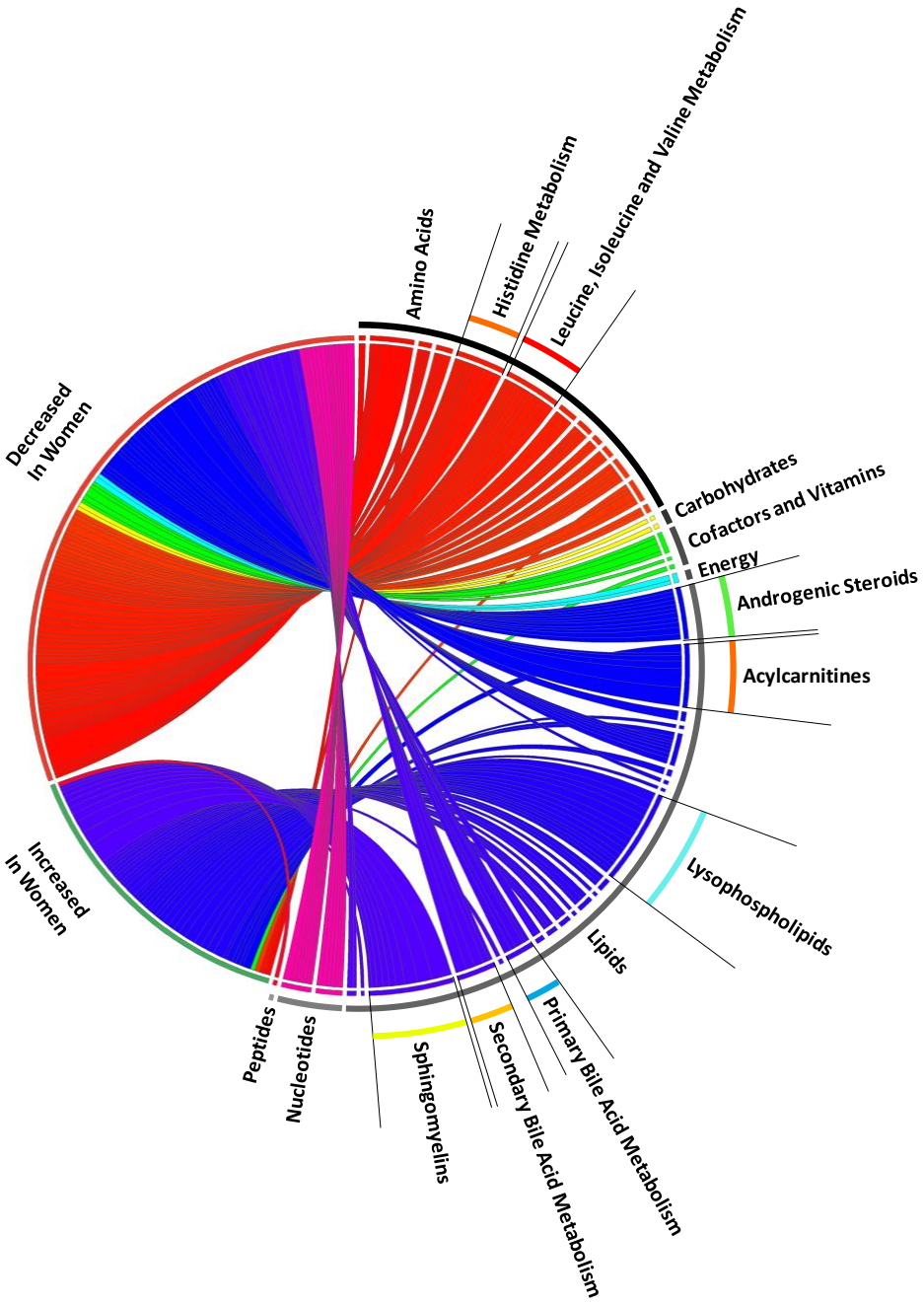

Day 0
Day 3
Day 7
[Tyrosine Metabolism] N-acetyltyrosine
[Tyrosine Metabolism] 3-(4-hydroxyphenyl)lactate
[Secondary Bile Acid Metabolism] taurolithocholate 3-sulfate
[Secondary Bile Acid Metabolism] taurocholenate sulfate
[Secondary Bile Acid Metabolism] glycocholenate sulfate
[Pyrimidine Metabolism] orotidine
[Pyrimidine Metabolism] 3-methylcytidine
[Primary Bile Acid Metabolism] glycochenodeoxycholate sulfate
[Primary Bile Acid Metabolism] glycochenodeoxycholate glucuronide
[Pregnenolone Steroids] pregnen-diol disulfate
[Lysine Metabolism] N6,N6,N6-trimethyllysine
[Leucine, Isoleucine and Valine Metabolism] beta-hydroxyisovalerate
[Leucine, Isoleucine and Valine Metabolism] 3-hydroxy-2-ethylpropionate
[Histidine Metabolism] N-acetylcarnosine
[Histidine Metabolism] formiminoglutamate
[Hemoglobin and Porphyrin Metabolism] bilirubin
[Guanidino and Acetamido Metabolism] 4-guanidinobutanoate
[Glutamate Metabolism] pyroglutamine
[Carnitine Metabolism] deoxycarnitine
[Androgenic Steroids] 5alpha-androstan-3beta,17beta-diol disulfate
[Androgenic Steroids] 5alpha-androstan-3beta,17alpha-diol disulfate
[Androgenic Steroids] 5alpha-androstan-3alpha,17beta-diol disulfate
[Acylcarnitine] tiglyl carnitine (C5)
[Acylcarnitine] succinylcarnitine (C4)
[Acylcarnitine] octadecanedioylcarnitine (C18-DC)
[Acylcarnitine] glutaroylcarnitine (C5)
[Acylcarnitine] 2-methylbutyroylcarnitine (C5)
−log10(P-value)
5
10
15
Effect Size Estimate
0.52
0.26
0.00
-0.26
-0.52

Figure 3.


Amino Acids
Histidine Metabolism
Leucine, Isoleucine and Valine Metabolism
Decreased In Women
Carbohydrates
Cofactors and Vitamins
Energy
Androgenic Steroids
Acylcarnitines
Lysophospholipids
Lipids
Primary Bile Acid Metabolism
Secondary Bile Acid Metabolism
Sphingomyelins
Nucleotides
Peptides
Increased In Women

Supplement

Supplemental Tables

Supplemental Figures

Supplemental Table 1. Additional Cohort Characteristics Stratified by Sex

Characteristic	Female	Male	Total
N	**151**	**277**	**428**
Admission Type			
Brain surgery No. (%)	2 (1.3)	2 (0.7)	4 (0.9)
Cardiac surgery No. (%)	31 (20.5)	50 (18.1)	81 (18.9)
Cardiovascular No. (%)	15 (9.9)	36 (13.0)	51 (11.9)
Gastrointestinal/liver No. (%)	5 (3.3)	9 (3.3)	14 (3.3)
Hematologic/oncologic No. (%)	0 (0)	1 (0.4)	1 (0.2)
Metabolic/Renal No. (%)	0 (0)	8 (2.9)	8 (1.9)
Neurologic No. (%)	132 (30.3)	169 (21.3)	301 (24.5)
Other non-operative No. (%)	2 (0.5)	6 (0.8)	8 (0.7)
Other operative No. (%)	17 (3.9)	17 (2.1)	34 (2.8)
Respiratory No. (%)	16 (10.6)	24 (8.7)	40 (9.4)
Sepsis/infectious No. (%)	11 (7.3)	24 (8.7)	35 (8.2)
Thoracic surgery No. (%)	5 (3.3)	8 (2.9)	13 (3.0)
Transplantation No. (%)	3 (2.0)	10 (3.6)	13 (3.0)
Trauma No. (%)	6 (4.0)	32 (11.6)	38 (8.9)
Vascular No. (%)	4 (2.7)	7 (2.5)	11 (2.6)

Supplemental Table 2. Additional Cohort Characteristics Stratified by Subjects Included and Excluded in the Analytic Cohort

Characteristic	Included Total	Excluded Total	P-value
No.	**428**	**25**	
Female Gender No. (%)	**151 (35.3)**	**6 (24.0)**	**0.25**
Age years Mean (SD)	**64.2 (14.9)**	**72.0 (10.5)**	**0.01***
Day 0 25(OH)D ng/ml Mean (SD)	**13.9 (8.8)**	**13.0 (6.3)**	**0.58***
SAPS II Mean (SD)	**33.4 (15.4)**	**35.6 (16.1)**	**0.49***
Day 0 C-reactive protein µg/mL Mean (SD)	**124.9 (89.8)**	**140.4 (75.7)**	**0.40***
Day 0 Procalcitonin ng/ml Median [IQR]	**0.66 [0.17, 2.79]**	**0.85 [0.47, 2.21]**	**0.26†**
Vitamin D_3 Intervention No. (%)	**212 (49.53)**	**13 (52.0)**	**0.81**
ICU			**0.18**
Anesthesia ICU No. (%)	**83 (19.4)**	**3 (12.0)**	
Cardiac Surgery ICU No. (%)	**126 (29.4)**	**8 (32.0)**	
Surgical ICU No. (%)	**23 (5.4)**	**1 (4.0)**	
Medicine ICU No. (%)	**90 (21.0)**	**10 (40.0)**	
Neurological ICU No. (%)	**106 (24.8)**	**3 (12.0)**	
3-day Mortality No. (%)	**11 (2.6)**	**15 (60.0)**	**<0.001**
7-day Mortality No. (%)	**24 (5.6)**	**17 (68.0)**	**<0.001**
28-day Mortality No. (%)	**95 (22.2)**	**19 (76.0)**	**<0.001**

Note: Data presented as n (%) unless otherwise indicated. P values determined by chi-square unless designated by (*) then P value determined by ANOVA or by (†) determined by Kruskal-Wallis test.

Supplemental Table 3. At randomization OPLS-DA model goodness of fit, predictive ability and model significance

	OPLS-DA			**Permutation (*n* = 200)**		**CV-ANOVA**
Classification Model	R2X	R2Y	Q2	R2 intercept (*x*-axis, *y*-axis)	Q2 intercept (*x*-axis, *y*-axis)	P-Value
Sex	0.341	1.00	0.423	0.00, 0.385	0.00, -0.387	<0.001

Supplemental Table 4. At randomization (Day 0) metabolite differences in Women relative to Men

Metabolite	Fold Change	FDR adjusted P-value	-log(10)p	Super Pathway	Sub Pathway
N-acetylglycine	2.65	3.74 E-06	5.43	Amino Acid	Glycine, Serine and Threonine Metabolism
1-palmitoyl-2-oleoyl-GPI (16:0/18:1)	2.26	2.34 E-06	5.63	Lipid	Phosphatidylinositol
Sphinganine-1-phosphate	2.52	1.01 E-07	6.99	Lipid	Sphingolipid Synthesis
Sphingomyelin (d18:1/25:0, d19:0/24:1, d20:1/23:0, d19:1/24:0)	3.07	5.89 E-10	9.23	Lipid	Sphingomyelins
Sphingomyelin (d17:1/14:0, d16:1/15:0)	2.89	5.89 E-10	9.23	Lipid	Sphingomyelins
Sphingomyelin (d18:2/14:0, d18:1/14:1)	2.66	7.51 E-08	7.12	Lipid	Sphingomyelins
Sphingomyelin (d18:2/21:0, d16:2/23:0)	2.06	3.22 E-05	4.49	Lipid	Sphingomyelins
N-acetylcarnosine	0.44	1.61 E-07	6.79	Amino Acid	Histidine Metabolism
Pyridoxate	0.40	1.41 E-09	8.85	Cofactors and Vitamins	Vitamin B6 Metabolism
5alpha-androstan-3beta,17beta-diol disulfate	0.39	1.23 E-09	8.91	Lipid	Androgenic Steroids
5alpha-androstan-3alpha,17beta-diol disulfate	0.38	1.23 E-09	8.92	Lipid	Androgenic Steroids
Taurocholate	0.37	4.20 E-06	5.38	Lipid	Primary Bile Acid Metabolism

Note: Fold change greater than 1 indicates an increase in metabolite in women relative to men.

Supplemental Table 5. Metabolites significantly Increased in Women relative to Men over time

Metabolite	P-value	Bonferroni corrected P-value	-log10p	β Coefficient	Super Pathway	Sub Pathway
Creatine	8.96 E-10	5.18 E-07	9.05	0.17	Amino Acid	Creatine Metabolism
N-acetylglycine	1.02 E-07	5.87 E-05	6.99	0.15	Amino Acid	Glycine, Serine and Threonine Metabolism
Thyroxine	7.27 E-08	4.20 E-05	7.14	0.14	Amino Acid	Tyrosine Metabolism
Beta-cryptoxanthin	7.71 E-07	4.46 E-04	6.11	0.17	Cofactors and Vitamins	Vitamin A Metabolism
Androstenediol (3beta,17beta) monosulfate	3.82 E-12	2.21 E-09	11.42	0.24	Lipid	Androgenic Steroids
N-behenoyl-sphingadienine (d18:2/22:0)	1.95 E-05	1.13 E-02	4.71	0.11	Lipid	Ceramides
Propionylglycine (C3)	5.61 E-06	3.24 E-03	5.25	0.14	Lipid	Fatty Acid Metabolism
Hexanoylglycine (C6)	2.81 E-07	1.63 E-04	6.55	0.17	Lipid	Fatty Acid Metabolism
Glycerol	7.83 E-10	4.53 E-07	9.11	0.19	Lipid	Glycerolipid Metabolism
Pentadecanoate (15:0)	3.99 E-05	2.31 E-02	4.40	0.10	Lipid	Long Chain Fatty Acid
1-linoleoyl-GPE (18:2)	3.71 E-15	2.14 E-12	14.43	0.21	Lipid	Lysophospholipid
1-palmitoyl-GPA (16:0)	1.77 E-11	1.02 E-08	10.75	0.20	Lipid	Lysophospholipid
1-linoleoyl-GPA (18:2)	5.69 E-10	3.29 E-07	9.24	0.20	Lipid	Lysophospholipid
1-linolenoyl-GPC (18:3)	1.57 E-11	9.08 E-09	10.80	0.20	Lipid	Lysophospholipid
1-stearoyl-GPE (18:0)	6.74 E-16	3.90 E-13	15.17	0.19	Lipid	Lysophospholipid
1-palmitoleoyl-GPC (16:1)	5.95 E-11	3.44 E-08	10.23	0.17	Lipid	Lysophospholipid
1-arachidonoyl-GPE (20:4n6)	2.74 E-10	1.58 E-07	9.56	0.16	Lipid	Lysophospholipid
2-stearoyl-GPE (18:0)	2.13 E-09	1.23 E-06	8.67	0.16	Lipid	Lysophospholipid
1-oleoyl-GPE (18:1)	5.81 E-10	3.36 E-07	9.24	0.16	Lipid	Lysophospholipid
2-palmitoyl-GPC (16:0)	7.59 E-09	4.39 E-06	8.12	0.15	Lipid	Lysophospholipid
1-lignoceroyl-GPC (24:0)	3.89 E-06	2.25 E-03	5.41	0.15	Lipid	Lysophospholipid
1-linoleoyl-GPI (18:2)	9.30 E-08	5.37 E-05	7.03	0.14	Lipid	Lysophospholipid
1-arachidonoyl-GPC (20:4)	8.26 E-07	4.78 E-04	6.08	0.13	Lipid	Lysophospholipid
1-(1-enyl-oleoyl)-GPE (P-18:1)	1.09 E-06	6.28 E-04	5.96	0.15	Lipid	Lysoplasmalogen
1-(1-enyl-palmitoyl)-GPE (P-16:0)	7.38 E-07	4.26 E-04	6.13	0.14	Lipid	Lysoplasmalogen
1-(1-enyl-palmitoyl)-GPC (P-16:0)	1.02 E-06	5.91 E-04	5.99	0.14	Lipid	Lysoplasmalogen
1-(1-enyl-stearoyl)-GPE (P-18:0)	5.23 E-06	3.02 E-03	5.28	0.13	Lipid	Lysoplasmalogen
1-dihomo-linolenylglycerol (20:3)	1.34 E-05	7.72 E-03	4.87	0.16	Lipid	Monoacylglycerol
1-palmitoleoyl-2-linolenoyl-GPC (16:1/18:3)	1.60 E-09	9.27 E-07	8.79	0.20	Lipid	Phosphatidylcholine (PC)
1-myristoyl-2-arachidonoyl-GPC (14:0/20:4)	3.85 E-08	2.23 E-05	7.41	0.16	Lipid	Phosphatidylcholine (PC)
1-linoleoyl-2-linolenoyl-GPC (18:2/18:3)	9.11 E-06	5.27 E-03	5.04	0.15	Lipid	Phosphatidylcholine (PC)
Glycerophosphorylcholine	5.89 E-06	3.40 E-03	5.23	0.12	Lipid	Phospholipid Metabolism
1-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2)	1.98 E-07	1.14 E-04	6.70	0.13	Lipid	Plasmalogen
1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (P-16:0/18:2)	3.05 E-05	1.76 E-02	4.52	0.12	Lipid	Plasmalogen
1-(1-enyl-stearoyl)-2-oleoyl-GPE (P-18:0/18:1)	6.92 E-06	4.00 E-03	5.16	0.11	Lipid	Plasmalogen
Linolenate (18:3n3 or 3n6)	1.02 E-06	5.91 E-04	5.99	0.16	Lipid	Polyunsaturated Fatty Acid
Hexadecadienoate (16:2n6)	2.18 E-05	1.26 E-02	4.66	0.14	Lipid	Polyunsaturated Fatty Acid
Linoleate (18:2n6)	4.41 E-05	2.55 E-02	4.36	0.12	Lipid	Polyunsaturated Fatty Acid
5alpha-pregnan-3beta,20alpha-diol monosulfate	1.19 E-06	6.86 E-04	5.93	0.16	Lipid	Progestin Steroids
Sphingomyelin (d18:2/14:0, d18:1/14:1)	6.93 E-29	4.01 E-26	28.16	0.28	Lipid	Sphingomyelins
Sphingomyelin (d17:2/16:0, d18:2/15:0)	1.33 E-21	7.70 E-19	20.88	0.26	Lipid	Sphingomyelins
Sphingomyelin (d17:1/14:0, d16:1/15:0)	1.92 E-18	1.11 E-15	17.72	0.24	Lipid	Sphingomyelins
Sphingomyelin (d18:2/21:0, d16:2/23:0)	4.11 E-13	2.38 E-10	12.39	0.18	Lipid	Sphingomyelins
Sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)	3.76 E-10	2.17 E-07	9.43	0.17	Lipid	Sphingomyelins

Supplemental Table 5. Metabolites significantly Increased in Women relative to Men over time (Continued)

Metabolite	P-value	Bonferroni corrected P-value	-log10p	β Coefficient	Super Pathway	Sub Pathway
Sphingomyelin (d18:1/19:0, d19:1/18:0)	1.28 E-09	7.38 E-07	8.89	0.15	Lipid	Sphingomyelins
Sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)	2.74 E-09	1.58 E-06	8.56	0.15	Lipid	Sphingomyelins
Tricosanoyl sphingomyelin (d18:1/23:0)	5.28 E-08	3.05 E-05	7.28	0.13	Lipid	Sphingomyelins
Sphingomyelin (d18:1/25:0, d19:0/24:1, d20:1/23:0, d19:1/24:0)	4.60 E-05	2.66 E-02	4.34	0.11	Lipid	Sphingomyelins
Hexadecasphingosine (d16:1)	3.29 E-05	1.90 E-02	4.48	0.13	Lipid	Sphingosines
Gamma-glutamylglycine	2.15 E-06	1.24 E-03	5.67	0.13	Peptide	Gamma-glutamyl Amino Acid

Note: Significant results presented following individual mixed effects modeling of each of the 578 individual metabolites measured at day 0, 3 and 7. All estimates adjusted for age, Simplified Acute Physiology Score (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 $P < 8.65 \times 10^{-5}$ was used to identify all significant associations. GPC is Glycerophosphorylcholine; GPE is glycerophosphoethanolamine.

Supplemental Table 6. Metabolites significantly decreased in Women relative to Men over time

Metabolite	P-value value	Bonferroni corrected P-value	-log10p	β Coefficient	Super Pathway	Sub Pathway
Alpha-ketoglutaramate	2.67 E-06	1.54 E-03	5.57	-0.13	Amino Acid	Glutamate Metabolism
N-acetyl-aspartyl-glutamate	4.83 E-09	2.79 E-06	8.32	-0.20	Amino Acid	Glutamate Metabolism
Pyroglutamine	9.68 E-48	5.60 E-45	47.01	-0.47	Amino Acid	Glutamate Metabolism
N-acetylserine	2.56 E-05	1.48 E-02	4.59	-0.11	Amino Acid	Glycine, Serine and Threonine Metabolism
N-acetylthreonine	3.64 E-07	2.11 E-04	6.44	-0.13	Amino Acid	Glycine, Serine and Threonine Metabolism
Guanidinosuccinate	3.62 E-06	2.09 E-03	5.44	-0.20	Amino Acid	Guanidino and Acetamido Metabolism
1-methylguanidine	2.17 E-07	1.25 E-04	6.66	-0.24	Amino Acid	Guanidino and Acetamido Metabolism
4-guanidinobutanoate	6.80 E-12	3.93 E-09	11.17	-0.27	Amino Acid	Guanidino and Acetamido Metabolism
1-ribosyl-imidazoleacetate	3.75 E-05	2.17 E-02	4.43	-0.14	Amino Acid	Histidine Metabolism
Imidazole lactate	2.43 E-07	1.40 E-04	6.61	-0.16	Amino Acid	Histidine Metabolism
1-methylhistidine	4.93 E-07	2.85 E-04	6.31	-0.16	Amino Acid	Histidine Metabolism
3-methylhistidine	1.71 E-06	9.90 E-04	5.77	-0.21	Amino Acid	Histidine Metabolism
N-acetyl-1-methylhistidine	3.44 E-07	1.99 E-04	6.46	-0.22	Amino Acid	Histidine Metabolism
Formiminoglutamate	1.64 E-12	9.50 E-10	11.78	-0.26	Amino Acid	Histidine Metabolism
N-acetylcarnosine	2.99 E-28	1.73 E-25	27.52	-0.35	Amino Acid	Histidine Metabolism
N-acetylvaline	2.55 E-05	1.47 E-02	4.59	-0.10	Amino Acid	Leucine, Isoleucine and Valine Metabolism
N-acetylleucine	7.65 E-06	4.42 E-03	5.12	-0.12	Amino Acid	Leucine, Isoleucine and Valine Metabolism
Alpha-hydroxyisocaproate	9.27 E-07	5.36 E-04	6.03	-0.13	Amino Acid	Leucine, Isoleucine and Valine Metabolism
3-hydroxyisobutyrate	1.37 E-06	7.90 E-04	5.86	-0.13	Amino Acid	Leucine, Isoleucine and Valine Metabolism
3-hydroxy-2-ethylpropionate	2.06 E-09	1.19 E-06	8.69	-0.15	Amino Acid	Leucine, Isoleucine and Valine Metabolism
2-hydroxy-3-methylvalerate	5.00 E-08	2.89 E-05	7.30	-0.16	Amino Acid	Leucine, Isoleucine and Valine Metabolism
2,3-dihydroxy-2-methylbutyrate	4.77 E-07	2.76 E-04	6.32	-0.17	Amino Acid	Leucine, Isoleucine and Valine Metabolism
Beta-hydroxyisovalerate	4.04 E-12	2.33 E-09	11.39	-0.19	Amino Acid	Leucine, Isoleucine and Valine Metabolism
N6,N6,N6-trimethyllysine	3.62 E-13	2.09 E-10	12.44	-0.20	Amino Acid	Lysine Metabolism
Cysteine	5.32 E-06	3.07 E-03	5.27	-0.12	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
5-methylthioribose	3.22 E-05	1.86 E-02	4.49	-0.15	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
S-adenosylhomocysteine	2.23 E-07	1.29 E-04	6.65	-0.16	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
Lanthionine	7.83 E-07	4.53 E-04	6.11	-0.17	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
Phenyllactate	2.48 E-08	1.43 E-05	7.61	-0.18	Amino Acid	Phenylalanine Metabolism
N-acetylphenylalanine	7.43 E-07	4.29 E-04	6.13	-0.18	Amino Acid	Phenylalanine Metabolism
Acisoga	1.09 E-05	6.28 E-03	4.96	-0.15	Amino Acid	Polyamine Metabolism
(N(1) + N(8))-acetylspermidine	6.12 E-07	3.53 E-04	6.21	-0.16	Amino Acid	Polyamine Metabolism
N-acetyl-isoputreanine	3.87 E-06	2.24 E-03	5.41	-0.16	Amino Acid	Polyamine Metabolism
Indole-3-carboxylate	4.60 E-06	2.66 E-03	5.34	-0.13	Amino Acid	Tryptophan Metabolism
Kynurenine	5.46 E-08	3.16 E-05	7.26	-0.14	Amino Acid	Tryptophan Metabolism
Indoleacetate	3.92 E-06	2.27 E-03	5.41	-0.16	Amino Acid	Tryptophan Metabolism
Picolinate	2.51 E-07	1.45 E-04	6.60	-0.20	Amino Acid	Tryptophan Metabolism
N-acetylkynurenine	3.00 E-06	1.74 E-03	5.52	-0.22	Amino Acid	Tryptophan Metabolism
2-hydroxyphenylacetate	6.82 E-06	3.94 E-03	5.17	-0.16	Amino Acid	Tyrosine Metabolism
3-(4-hydroxyphenyl)lactate	6.05 E-12	3.50 E-09	11.22	-0.21	Amino Acid	Tyrosine Metabolism

Supplemental Table 6. Metabolites significantly decreased in Women relative to Men over time (Continued)

Metabolite	P-value value	Bonferroni corrected P-value	-log10p	β Coefficient	Super Pathway	Sub Pathway
N-acetyltyrosine	4.63 E-09	2.68 E-06	8.33	-0.23	Amino Acid	Tyrosine Metabolism
Argininate	4.04 E-06	2.34 E-03	5.39	-0.14	Amino Acid	Urea cycle; Arginine and Proline Metabolism
Urea	2.39 E-07	1.38 E-04	6.62	-0.14	Amino Acid	Urea cycle; Arginine and Proline Metabolism
Glucuronate	2.27 E-06	1.31 E-03	5.64	-0.18	Carbohydrate	Aminosugar Metabolism
Sedoheptulose	2.79 E-07	1.61 E-04	6.55	-0.16	Carbohydrate	Pentose Metabolism
Biliverdin	1.64 E-05	9.48 E-03	4.79	-0.14	Cofactors and Vitamins	Hemoglobin and Porphyrin Metabolism
Bilirubin (E,Z or Z,E)	2.07 E-08	1.20 E-05	7.68	-0.18	Cofactors and Vitamins	Hemoglobin and Porphyrin Metabolism
Bilirubin	2.50 E-12	1.44 E-09	11.60	-0.20	Cofactors and Vitamins	Hemoglobin and Porphyrin Metabolism
D-urobilin	2.26 E-06	1.31 E-03	5.65	-0.20	Cofactors and Vitamins	Hemoglobin and Porphyrin Metabolism
Nicotinamide riboside	2.80 E-05	1.62 E-02	4.55	-0.14	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism
2-methylcitrate/homocitrate	3.79 E-06	2.19 E-03	5.42	-0.18	Energy	TCA Cycle
Androstenediol (3beta,17beta) disulfate	1.85 E-08	1.07 E-05	7.73	-0.18	Lipid	Androgenic Steroids
5alpha-androstan-3beta,17alpha-diol disulfate	1.88 E-11	1.09 E-08	10.73	-0.27	Lipid	Androgenic Steroids
5alpha-androstan-3beta,17beta-diol disulfate	3.92 E-27	2.27 E-24	26.41	-0.36	Lipid	Androgenic Steroids
5alpha-androstan-3alpha,17beta-diol disulfate	2.00 E-27	1.16 E-24	26.70	-0.46	Lipid	Androgenic Steroids
Deoxycarnitine	8.43 E-14	4.87 E-11	13.07	-0.19	Lipid	Carnitine Metabolism
Propionylcarnitine (C3)	6.88 E-05	3.98 E-02	4.16	-0.12	Lipid	Acylcarnitine
Malonylcarnitine (C3-DC)	8.56 E-07	4.95 E-04	6.07	-0.14	Lipid	Acylcarnitine
Succinylcarnitine (C4)	1.22 E-08	7.07 E-06	7.91	-0.17	Lipid	Acylcarnitine
2-methylmalonylcarnitine (C4-DC)	1.02 E-07	5.89 E-05	6.99	-0.20	Lipid	Acylcarnitine
Isovalerylcarnitine (C5)	1.49 E-06	8.59 E-04	5.83	-0.16	Lipid	Acylcarnitine
2-methylbutyroylcarnitine (C5)	3.91 E-11	2.26 E-08	10.41	-0.23	Lipid	Acylcarnitine
Tiglyl carnitine (C5)	7.31 E-14	4.23 E-11	13.14	-0.24	Lipid	Acylcarnitine
Glutaroylcarnitine (C5)	7.32 E-15	4.23 E-12	14.14	-0.24	Lipid	Acylcarnitine
3-Methyladipoylcarnitine (C7-DC)	5.51 E-07	3.18 E-04	6.26	-0.18	Lipid	Acylcarnitine
Octanoylcarnitine (C8)	1.55 E-06	8.96 E-04	5.81	-0.16	Lipid	Acylcarnitine
Cis-4-decenoylcarnitine (C10:1)	1.29 E-06	7.48 E-04	5.89	-0.15	Lipid	Acylcarnitine
Decanoylcarnitine (C10)	2.19 E-08	1.27 E-05	7.66	-0.18	Lipid	Acylcarnitine
Laurylcarnitine (C12)	6.99 E-07	4.04 E-04	6.16	-0.15	Lipid	Acylcarnitine
Octadecanedioylcarnitine (C18-DC)	3.26 E-10	1.88 E-07	9.49	-0.22	Lipid	Acylcarnitine
Octadecenedioylcarnitine (C18:1-DC)	1.05 E-08	6.08 E-06	7.98	-0.25	Lipid	Acylcarnitine
N-acetyl-2-aminooctanoate	3.61 E-05	2.08 E-02	4.44	-0.15	Lipid	Fatty Acid, Amino
Hydroxy-3-carboxy-4-methyl-5-propyl-2-furanpropanoate	6.74 E-05	3.90 E-02	4.17	-0.14	Lipid	Fatty Acid, Dicarboxylate
2-hydroxyadipate	1.97 E-05	1.14 E-02	4.71	-0.17	Lipid	Fatty Acid, Dicarboxylate
Heptenedioate (C7:1-DC)	1.55 E-06	8.95 E-04	5.81	-0.18	Lipid	Fatty Acid, Dicarboxylate
3-methyladipate	8.00 E-07	4.63 E-04	6.10	-0.19	Lipid	Fatty Acid, Dicarboxylate
3-carboxy-4-methyl-5-propyl-2-furanpropanoate	5.59 E-09	3.23 E-06	8.25	-0.22	Lipid	Fatty Acid, Dicarboxylate
Myo-inositol	3.90 E-06	2.26 E-03	5.41	-0.16	Lipid	Inositol Metabolism
Pregnen-diol disulfate	5.80 E-13	3.35 E-10	12.24	-0.23	Lipid	Pregnenolone Steroids
Glycochenodeoxycholate	1.59 E-06	9.18 E-04	5.80	-0.20	Lipid	Primary Bile Acid Metabolism
Taurocholate	4.19 E-05	2.42 E-02	4.38	-0.21	Lipid	Primary Bile Acid Metabolism

Supplemental Table 6. Metabolites significantly decreased in Women relative to Men over time (Continued)

Metabolite	P-value value	Bonferroni corrected P-value	-log10p	β Coefficient	Super Pathway	Sub Pathway
Glycochenodeoxycholate glucuronide	2.94 E-12	1.70 E-09	11.53	-0.28	Lipid	Primary Bile Acid Metabolism
Taurochenodeoxycholate	2.20 E-08	1.27 E-05	7.66	-0.28	Lipid	Primary Bile Acid Metabolism
Glycochenodeoxycholate sulfate	4.26 E-11	2.46 E-08	10.37	-0.32	Lipid	Primary Bile Acid Metabolism
Glycodeoxycholate sulfate	4.01 E-06	2.32 E-03	5.4	-0.23	Lipid	Secondary Bile Acid Metabolism
Glycolithocholate sulfate	5.82 E-09	3.36 E-06	8.24	-0.27	Lipid	Secondary Bile Acid Metabolism
Glycocholenate sulfate	6.22 E-14	3.59 E-11	13.21	-0.28	Lipid	Secondary Bile Acid Metabolism
Taurolithocholate 3-sulfate	3.11 E-12	1.80 E-09	11.51	-0.34	Lipid	Secondary Bile Acid Metabolism
Taurocholenate sulfate	1.03 E-16	5.94 E-14	15.99	-0.38	Lipid	Secondary Bile Acid Metabolism
3beta,7alpha-dihydroxy-5-cholestenoate	1.57 E-05	9.10 E-03	4.8	-0.12	Lipid	Sterol
3beta-hydroxy-5-cholestenoate	7.45 E-09	4.30 E-06	8.13	-0.16	Lipid	Sterol
Urate	1.95 E-07	1.13 E-04	6.71	-0.13	Nucleotide	Purine Metabolism
N1-methylinosine	1.89 E-07	1.09 E-04	6.72	-0.18	Nucleotide	Purine Metabolism
Xanthosine	1.99 E-07	1.15 E-04	6.7	-0.18	Nucleotide	Purine Metabolism
Adenosine	7.16 E-07	4.14 E-04	6.15	-0.15	Nucleotide	Purine Metabolism
N6-carbamoylthreonyladenosine	1.34 E-06	7.72 E-04	5.87	-0.15	Nucleotide	Purine Metabolism
N2,N2-dimethylguanosine	3.68 E-05	2.13 E-02	4.43	-0.14	Nucleotide	Purine Metabolism
3-methylcytidine	1.84 E-06	1.06 E-03	5.73	-0.13	Nucleotide	Pyrimidine Metabolism
Orotate	2.34 E-05	1.35 E-02	4.63	-0.15	Nucleotide	Pyrimidine Metabolism
Dihydroorotate	9.20 E-06	5.32 E-03	5.04	-0.16	Nucleotide	Pyrimidine Metabolism
Orotidine	7.51 E-12	4.34 E-09	11.12	-0.37	Nucleotide	Pyrimidine Metabolism
5,6-dihydrothymine	4.93 E-05	2.85 E-02	4.31	-0.11	Nucleotide	Pyrimidine Metabolism
Pseudouridine	4.45 E-05	2.57 E-02	4.35	-0.1	Nucleotide	Pyrimidine Metabolism
3-ureidopropionate	6.93 E-07	4.00 E-04	6.16	-0.19	Nucleotide	Pyrimidine Metabolism

Note: Significant results presented following individual mixed effects modeling of each of the 578 individual metabolites measured at day 0, 3 and 7. All estimates adjusted for age, Simplified Acute Physiology Score (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 $P < 8.65 \times 10^{-5}$ was used to identify all significant associations. For the Acylcarnitine 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). DC following the carbon number is a dicarboxylic acylcarnitine. GPE is glycerophosphoethanolamine.

Supplemental Table 7. Metabolites differentially associated in Women and Men with 28-day mortality over time

Metabolite	Bonferroni corrected P-value in women	Odds Ratio for 28-day mortality in women	Bonferroni corrected P-value in men	Odds Ratio for 28-day mortality in men	Super Pathway	Sub Pathway
Creatine	1.00	2.48	**3.67 E-06**	2.99	Amino Acid	Creatine Metabolism
N-acetylglutamine	4.98 E-01	2.28	**4.53 E-04**	2.44	Amino Acid	Glutamate Metabolism
cys-gly, oxidized	**1.59 E-04**	4.02	1.00	1.45	Amino Acid	Glutathione Metabolism
N-acetylhistidine	1.00	1.93	**2.65 E-07**	3.60	Amino Acid	Histidine Metabolism
1-methylhistidine	**9.88 E-03**	2.72	1.00	1.52	Amino Acid	Histidine Metabolism
1-ribosyl-imidazoleacetate	1.00	1.43	**4.72 E-03**	2.13	Amino Acid	Histidine Metabolism
Ethylmalonate	**3.36 E-05**	4.03	6.54 E-01	1.85	Amino Acid	Leucine, Isoleucine and Valine Metabolism
2,3-dihydroxy-2-methylbutyrate	**2.78 E-03**	3.53	1.00	1.63	Amino Acid	Leucine, Isoleucine and Valine Metabolism
3-hydroxyisobutyrate	**6.05 E-03**	3.56	7.74 E-01	1.80	Amino Acid	Leucine, Isoleucine and Valine Metabolism
Isovalerylglycine	7.20 E-02	1.98	**1.40 E-06**	2.43	Amino Acid	Leucine, Isoleucine and Valine Metabolism
N,N,N-trimethyl-5-aminovalerate	**9.67 E-04**	3.45	1.00	1.72	Amino Acid	Lysine Metabolism
Pipecolate	1.00	1.99	**2.30 E-03**	2.33	Amino Acid	Lysine Metabolism
Lanthionine	5.26 E-01	1.94	**5.72 E-04**	2.22	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
N-acetylputrescine	4.38 E-01	2.34	**9.93 E-08**	3.32	Amino Acid	Polyamine Metabolism
Prolylhydroxyproline	1.00	1.05	**1.17 E-02**	2.61	Amino Acid	Urea cycle; Arginine and Proline Metabolism
N-acetylcitrulline	1.00	1.72	**5.64 E-06**	2.21	Amino Acid	Urea cycle; Arginine and Proline Metabolism
N-acetylglucosaminylasparagine	**1.03 E-03**	3.43	1.00	1.41	Carbohydrate	Aminosugar Metabolism
Ribulonate	**3.67 E-03**	2.78	1.00	1.54	Carbohydrate	Pentose Metabolism
1-methylnicotinamide	1.00	1.53	**1.40 E-09**	3.17	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism
Nicotinamide riboside	1.00	1.95	**4.60 E-12**	3.47	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism
N1-Methyl-2-pyridon E-5-carboxamide	1.00	2.01	**1.82 E-05**	2.89	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism
Isobutyrylcarnitine (C4)	**3.22 E-06**	3.77	1.25 E-01	1.66	Lipid	Acylcarnitine
Glutaroylcarnitine (C5)	**1.34 E-03**	3.26	1.89 E-01	1.89	Lipid	Acylcarnitine
Adipoylcarnitine (C6-DC)	**2.13 E-05**	3.31	1.00	1.32	Lipid	Acylcarnitine
3-methyladipoylcarnitine (C7-DC)	**1.56 E-04**	3.26	1.00	1.33	Lipid	Acylcarnitine
Suberoylcarnitine (C8-DC)	**1.73 E-06**	3.23	1.00	1.44	Lipid	Acylcarnitine
Decanoylcarnitine (C10)	**2.02 E-02**	2.86	5.82 E-02	1.85	Lipid	Acylcarnitine
N-palmitoyl-heptadecasphingosine (d17:1/16:0)	1.00	1.27	**2.53 E-02**	2.34	Lipid	Ceramide
Glycosyl-N-(2-hydroxynervonoyl)-sphingosine (d18:1/24:1(2OH))	1.00	0.73	**9.41 E-03**	2.48	Lipid	Ceramide
Methylmalonate	**3.18 E-03**	3.25	9.20 E-02	1.99	Lipid	Fatty Acid Metabolism
9,10-DiHOME	**3.57 E-02**	2.49	5.92 E-02	1.84	Lipid	Fatty Acid Metabolism
Pregnen-diol disulfate	**5.27 E-05**	4.02	1.00	1.61	Lipid	Pregnenolone Steroid
Androstenediol (3beta,17beta) disulfate	**4.33 E-03**	3.16	1.00	1.46	Lipid	Androgenic Steroids
5alpha-pregnan-3beta,20alpha-diol disulfate	**3.85 E-03**	2.49	7.45 E-02	1.82	Lipid	Progestin Steroid
Glycochenodeoxycholate	1.00	1.52	**3.98 E-05**	1.93	Lipid	Primary Bile Acid Metabolism
Glycocholate	8.18 E-01	1.65	**6.96 E-05**	1.79	Lipid	Primary Bile Acid Metabolism
Tauroursodeoxycholate	1.00	1.44	**4.61 E-03**	1.62	Lipid	Secondary Bile Acid Metabolism

Supplemental Table 7. Metabolites Differentially associated in women and men with 28-day mortality over time (Continued)

Metabolite	Bonferroni corrected P-value in women	Odds Ratio for 28-day mortality in women	Bonferroni corrected P-value in men	Odds Ratio for 28-day mortality in men	Super Pathway	Sub Pathway
Glycodeoxycholate sulfate	5.36 E-02	1.91	**3.56 E-05**	1.71	Lipid	Secondary Bile Acid Metabolism
Glycolithocholate sulfate	1.00	1.64	**8.35 E-05**	1.77	Lipid	Secondary Bile Acid Metabolism
Taurolithocholate 3-sulfate	3.18 E-01	1.89	**1.42 E-07**	1.91	Lipid	Secondary Bile Acid Metabolism
Campesterol	1.00	1.13	**2.64 E-05**	3.40	Lipid	Sterol
Beta-sitosterol	9.46 E-02	2.20	**1.66 E-06**	2.73	Lipid	Sterol
Adenosine	**2.03 E-02**	2.97	1.00	1.68	Nucleotide	Purine Metabolism
3-methylcytidine	**1.43 E-02**	3.67	1.00	1.09	Nucleotide	Pyrimidine Metabolism
3-ureidopropionate	8.86 E-02	2.35	**7.46 E-08**	2.57	Nucleotide	Pyrimidine Metabolism

Note: Divergent results presented following individual mixed effects modeling of each of the 578 individual metabolites measured at day 0, 3 and 7 in women (N=151) and in men (N=277). All estimates adjusted for age, Simplified Acute Physiology Score (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 $P < 8.65 \times 10^{-5}$ (Bonferroni corrected P-value < 0.05) was used to identify all significant associations shown in bold. For the Acylcarnitine sub pathway: a capital C is followed by the number of carbons within the fatty acyl group attached to the carnitine. DC following the carbon number is a dicarboxylic acylcarnitine.

Supplemental Table 8. Day 3 Sex-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	**2.67 E-05**	1-stearoyl-GPE (18:0)	Lipid	Lysophospholipid	**6.74 E-16**	0.189
		2-stearoyl-GPE (18:0)	Lipid	Lysophospholipid	**2.13 E-09**	0.159
B	**1.54 E-05**	2-hydroxy-3-methylvalerate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	**5.00 E-08**	-0.155
		Phenyllactate	Amino Acid	Phenylalanine Metabolism	**2.48 E-08**	-0.175
		3-(4-hydroxyphenyl)lactate	Amino Acid	Tyrosine Metabolism	**6.05 E-12**	-0.213
		alpha-hydroxyisocaproate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	**9.27 E-07**	-0.129
		4-methyl-2-oxopentanoate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	6.33 E-03	-0.066
C	**1.15 E-05**	3-hydroxy-2-ethylpropionate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	**2.06 E-09**	-0.153
		beta-hydroxyisovalerate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	**4.04 E-12**	-0.188
D	**1.18 E-10**	5alpha-androstan-3alpha,17beta-diol disulfate	Lipid	Androgenic Steroids	**2.00 E-27**	-0.464
		5alpha-androstan-3beta,17beta-diol disulfate	Lipid	Androgenic Steroids	**3.92 E-27**	-0.364
E	**8.86 E-06**	androstenediol (3alpha, 17alpha) monosulfate	Lipid	Androgenic Steroids	1.49 E-04	-0.143
		Pregnen-diol disulfate	Lipid	Pregnenolone Steroids	**5.80 E-13**	-0.228
		androstenediol (3beta,17beta) disulfate	Lipid	Androgenic Steroids	**1.85 E-08**	-0.181
F	**1.20 E-08**	Glycochenodeoxycholate glucuronide	Lipid	Primary Bile Acid Metabolism	**2.94 E-12**	-0.277
		Glycochenodeoxycholate sulfate	Lipid	Primary Bile Acid Metabolism	**4.26 E-11**	-0.316
		Glycocholenate sulfate	Lipid	Secondary Bile Acid Metabolism	**6.22 E-14**	-0.277
		3beta-hydroxy-5-cholestenoate	Lipid	Sterol	**7.45 E-09**	-0.163
		3beta,7alpha-dihydroxy-5-cholestenoate	Lipid	Sterol	**1.57 E-05**	-0.123
		Taurocholenate sulfate	Lipid	Secondary Bile Acid Metabolism	**1.03 E-16**	-0.379
		Taurolithocholate 3-sulfate	Lipid	Secondary Bile Acid Metabolism	**3.11 E-12**	-0.337
G	**1.63 E-05**	Palmitoleoyl-arachidonoyl-glycerol (16:1/20:4)	Lipid	Diacylglycerol	**3.80 E-03**	0.102
		1-palmitoleoyl-2-linolenoyl-GPC (16:1/18:3)	Lipid	Phosphatidylcholine	**1.60 E-09**	0.197
		1-palmitoleoyl-GPC (16:1)	Lipid	Lysophospholipid	**5.95 E-11**	0.169
		1-linolenoyl-GPC (18:3)	Lipid	Lysophospholipid	**1.57 E-11**	0.195
		1-arachidonoyl-GPC (20:4)	Lipid	Lysophospholipid	**8.26 E-07**	0.126
		1-arachidonoyl-GPE (20:4n6)	Lipid	Lysophospholipid	**2.74 E-10**	0.162
		1-linoleoyl-GPE (18:2)	Lipid	Lysophospholipid	**3.71 E-15**	0.209

Note: Module P value is the Bonferroni adjusted P value of the GMM 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 578 individual metabolites measured at day 0, 3 and 7. All estimates adjusted for age, Simplified Acute Physiology Score (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 $P < 8.65 \times 10^{-5}$ was used to identify all significant associations.

Supplemental Table 9. Day 8 Sex-specific Metabolic Networks with similar effects via Gaussian graphical models

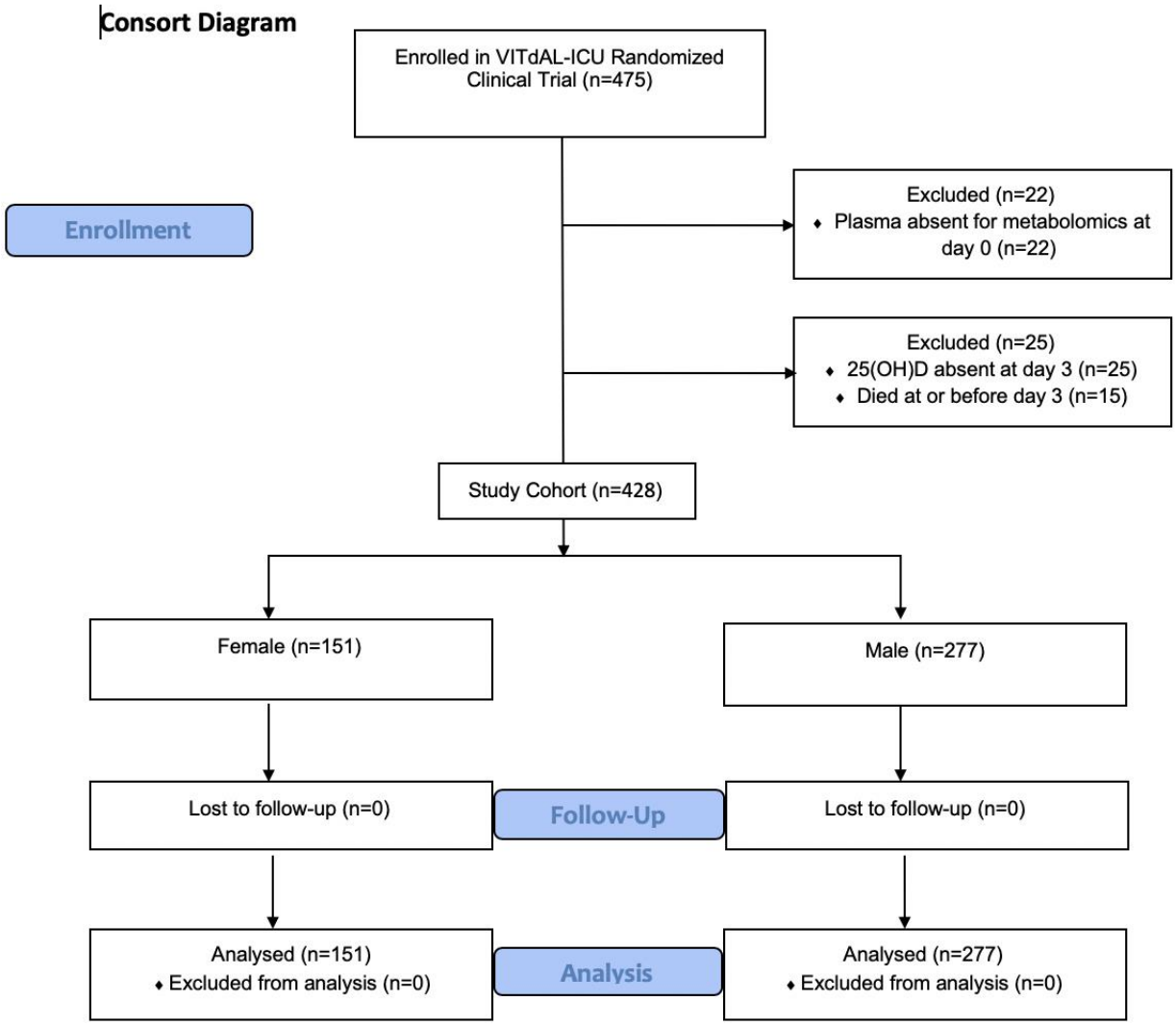
Module	Module p value	Metabolite	Super Pathway	Sub-pathway	Component p value	Component β Coefficient
H	**1.34 E-05**	2-hydroxy-3-methylvalerate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	**5.00 E-08**	-0.155
		alpha-hydroxyisocaproate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	**9.27 E-07**	-0.129
		Phenyllactate	Amino Acid	Phenylalanine Metabolism	**2.48 E-08**	-0.175
		3-(4-hydroxyphenyl)lactate	Amino Acid	Tyrosine Metabolism	**6.05 E-12**	-0.213
		4-methyl-2-oxopentanoate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	6.33 E-03	-0.066
		alpha-hydroxyisovalerate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	4.62 E-04	-0.098
		Phenylpyruvate	Amino Acid	Phenylalanine Metabolism	2.03 E-02	-0.066
I	**2.66 E-07**	3beta,7alpha-dihydroxy-5-cholestenoate	Lipid	Sterol	**1.57 E-05**	-0.123
		Glycolithocholate sulfate	Lipid	Secondary Bile Acid Metabolism	**5.82 E-09**	-0.275
		Taurolithocholate 3-sulfate	Lipid	Secondary Bile Acid Metabolism	**3.11 E-12**	-0.337
		Glycocholenate sulfate	Lipid	Secondary Bile Acid Metabolism	**6.22 E-14**	-0.277
		Taurocholenate sulfate	Lipid	Secondary Bile Acid Metabolism	**1.03 E-16**	-0.379
		Glycochenodeoxycholate glucuronide	Lipid	Primary Bile Acid Metabolism	**2.94 E-12**	-0.277
		Glycodeoxycholate sulfate	Lipid	Secondary Bile Acid Metabolism	**4.01 E-06**	-0.235
		3beta-hydroxy-5-cholestenoate	Lipid	Sterol	**7.45 E-09**	-0.163
		Taurodeoxycholate	Lipid	Secondary Bile Acid Metabolism	5.40 E-03	-0.131
		Glycochenodeoxycholate sulfate	Lipid	Primary Bile Acid Metabolism	**4.26 E-11**	-0.316
J	**4.93 E-05**	3-hydroxy-2-ethylpropionate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	**2.06 E-09**	-0.153
		3-hydroxyisobutyrate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	**1.37 E-06**	-0.134
		beta-hydroxyisovalerate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	**4.04 E-12**	-0.188
K	**2.27 E-05**	3-methylcytidine	Nucleotide	Pyrimidine Metabolism	**1.84 E-06**	-0.133
		Phenylacetate	Amino Acid	Phenylalanine Metabolism	3.43 E-02	-0.077
		Phenylacetylglutamine	Peptide	Acetylated Peptides	1.51 E-03	-0.119
L	**2.06 E-10**	5alpha-androstan-3alpha,17beta-diol disulfate	Lipid	Androgenic Steroids	**2.00 E-27**	-0.464
		5alpha-androstan-3beta,17beta-diol disulfate	Lipid	Androgenic Steroids	**3.92 E-27**	-0.364
M	**2.82 E-05**	1-linolenoyl-GPC (18:3)	Lipid	Lysophospholipid	**1.57 E-11**	0.195
		1-palmitoleoyl-GPC (16:1)	Lipid	Lysophospholipid	**5.95 E-11**	0.169
		Palmitoleoyl-linoleoyl-glycerol (16:1/18:2)	Lipid	Diacylglycerol	1.68 E-02	0.068
		Diacylglycerol (16:1/18:2 [2], 16:0/18:3 [1])	Lipid	Diacylglycerol	4.74 E-02	0.059
		Ceramide (d18:1/14:0, d16:1/16:0)	Lipid	Ceramides	3.26 E-02	0.058

		N-palmitoyl-heptadecasphingosine (d17:1/16:0)	Lipid	Ceramides	4.04 E-04	0.095

N	**1.33 E-06**	1-linoleoyl-GPA (18:2)	Lipid	Lysophospholipid	**5.69 E-10**	0.198
		1-palmitoyl-GPA (16:0)	Lipid	Lysophospholipid	**1.77 E-11**	0.205

Note: Module P value is the Bonferroni adjusted P value of the GMM 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 578 individual metabolites measured at day 0, 3 and 7. All estimates adjusted for age, Simplified Acute Physiology Score (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 $P < 8.65 \times 10^{-5}$ was used to identify all significant associations.

Supplemental Figure 1. Consort Diagram



Title: Sex-Specific response to high-dose vitamin D in Critical Illness: A post-hoc analysis of VITdAL-ICU Trial

Sowmya Chary, MBBS [1,6], Karin Amrein, MD, MSc [2], Jessica A. Lasky-Su, ScD [3], Sherif H. Mahmoud BSc (Pharm), MSc, PhD [4], Harald Dobnig, MD [5], Kenneth B. Christopher, MD, SM [3,6]

1. Harvard Medical School, Boston, USA
2. Division of Endocrinology and Diabetology, Medical University of Graz, Graz, Austria
3. Channing Division of Network Medicine, Brigham and Women's Hospital, Boston, USA
4. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta
5. Thyroid Endocrinology Osteoporosis Institute Dobnig, Graz, Austria
6. Division of Renal Medicine, Brigham and Women's Hospital, Boston, USA

Corresponding Author: Kenneth B. Christopher, MD, SM, Division of Renal Medicine, Brigham and Women's Hospital, 75 Francis Street, Boston, 02115 USA kbchristopher@bwh.harvard.edu p 617 272 0535

Author Contributions: Drs. Christopher, Amrein and Lasky-Su had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Chary, Amrein, Mahmoud, Lasky-Su, Christopher. Acquisition, analysis, or interpretation of data: All authors. Drafting of manuscript: All authors. Statistical analysis: Amrein, Mahmoud, Lasky-Su, Christopher. Obtained funding: Amrein, Dobnig, Christopher. Administrative technical or material support. All authors. Study Supervision: Amrein, Dobnig, Christopher.

Funding: The VITdAL-ICU trial was supported by the European Society for Clinical Nutrition and Metabolism (ESPEN), a research grant including provision of study medication from Fresenius Kabi (Germany), and the Austrian National Bank (Jubiläumsfonds, Project Nr. 14143). The metabolomics work was funded by the NIGMS/ R01 GM115774.

Running Head: Sex-specific response to High Dose Vitamin D

Total Word Count Body 2762

Abstract

Rationale: It is unclear if the metabolic response to high dose vitamin D in critical illness differs in women and men.

Objectives: To determine the sex-specific metabolomic response early in the course of critical illness.

Methods: We performed a post-hoc metabolomics study of the VITdAL-ICU randomized double-blind, placebo-controlled trial. Trial patients from Medical and Surgical Intensive Care Units at a tertiary university hospital with 25-hydroxyvitamin D level ≤ 20 ng/mL received either high dose oral vitamin D_3 (540,000 IU) or placebo. We performed an analysis of 578 metabolites in 1215 plasma samples from 428 subjects at randomization (day 0), day 3 and 7. Using mixed-effects modeling, we studied the association between sex-specific changes in individual metabolites over time and 25-hydroxyvitamin D response to intervention adjusted for age, Simplified Acute Physiology Score II, admission diagnosis and baseline 25-hydroxyvitamin D level. Additionally, we determined the sex-specific pharmacokinetics of high dose oral vitamin D.

Measurements and Main Results: 35% of subjects were women and 27% responded to high dose vitamin D_3, with an increase of ≥ 15 ng/ml 25-hydroxyvitamin D by day 3. Compared with women, the pharmacokinetic parameters of 25(OH)D using non-compartmental analysis showed significantly higher normalized AUC0-7 in men. At day 0, sex-specific metabolomic differences in patients who do or do not respond to vitamin D_3 are present. In the mixed-effects analysis, a sex-specific metabolomic response to vitamin D_3 is associated with differential metabolite patterns over time. Specifically, women have higher and more significant Bonferroni corrected

increases in circulating free fatty acids and long chain acylcarnitines than men in response to an increase of ≥ 15 ng/ml 25-hydroxyvitamin D by day 3.

Conclusions: Response to high dose oral vitamin D_3 is sex specific. Robust coordinated sex-specific metabolite differences are present with an increase in 25-hydroxyvitamin D by day 3.

Word count abstract: 299

Keywords:

Metabolite, Metabolomics, Sex, Gender, Critical Illness

Introduction

Women and men have dissimilar pharmacokinetics and pharmacodynamics in response to drug interventions (1). In metabolism, sex-specific genetic differences are present (2, 3). Sizable studies on circulating metabolites in ambulatory patients show notable sex-specific differences (2-4). Sex-specific pharmacological properties are poorly described, and most drugs do not have specific dosage recommendations for women and men (5-7).

Critically ill patients have substantial alterations in homeostasis as reflected by circulating metabolites. Blood based metabolomic studies early in critical illness can reflect dysregulated cellular byproducts, inform illness severity and predict outcomes (8, 9). Metabolomic profiles are shown to differ in critically ill patients with and without low 25(OH)D levels (10). A secondary outcome the VITdAL-ICU trial showed that in patients with low baseline vitamin D levels, mortality improved with high dose oral vitamin D_3 (11). Though the pharmacokinetics is well described in outpatients, the sex-specific effects of high dose vitamin D_3 in the critically ill is not known (12-15).

Metabolomics before and after intervention can furnish mechanistic insight into drug response and severity of critical illness (16). Sex-specific pharmacokinetics is likely present with oral medication use (17). But sex-specific pharmacometabolomics is poorly described especially in critical care. Therefore, we studied sex-specific differences in metabolic response in critically ill subjects following high dose oral vitamin D_3. We hypothesize that significant sex-specific pharmacokinetic and metabolomic responses to vitamin D intervention exist. We performed a

post-hoc metabolomics analysis of 1215 plasma samples from 428 patients collected during the VITdAL-ICU trial (11). We assessed the sex-specific changes in individual metabolites following high dose vitamin D intervention in critical illness. Secondly, we determined if sex-specific differences in pharmacokinetics or mediation exist in the relationship between the response to intervention and individual metabolite change.

Methods

The VITdAL-ICU trial randomized 475 critically ill adult patients with 25-hydroxyvitamin D [25(OH)D] < 20 ng/mL to vitamin D_3 or placebo given orally or via nasogastric tube once at a dose of 540,000 IU followed by 90,000 IU monthly (11). The loading dose of vitamin D_3 was dissolved in 45 mL of oleum arachidis (Oleovit D_3, Fresenius Kabi). Placebo group subjects received 45 mL of oleum arachidis (11). Blood samples were collected on days 0 (at randomization), 3 and 7. Plasma was fractionated, aliquoted and stored at -80°C. Four hundred fifty-three trial subjects had frozen plasma available for analysis. We excluded 25 trial subjects who did not have 25(OH)D measured at day 3 following randomization.

For determination of the pharmacokinetics of oral vitamin D_3 we utilized serum 25(OH)D levels, a marker of systemic vitamin D status (18). Chemiluminescence assay (IDS-iSYS, Immunodiagnostic Systems) was used to measure serum 25(OH)D. The assay coefficients of variation for control material were 13.4% at 13 ng/mL, 10% at 31 ng/mL, and 9.4% at 64 ng/mL (11). For pharmacokinetics evaluation, the area under the plasma concentration–time curve from vitamin D_3 dosing to day 7 (AUC0-7d) was calculated using the linear trapezoidal method. Patients with

missing 25(OH)D levels on day 3 or 7 and those that received placebo were excluded from the pharmacokinetics evaluation. AUC normalized to vitamin D_3 dose and body weight (AUCnorm) was calculated by dividing AUC 0-7d by dose in IU per kg body weight. Median AUCnorm and serum 25(OH)D levels on days 0, 3 and 7 values were compared between males and females.

VITdAL-ICU trial subject plasma aliquots were shipped on dry-ice to Metabolon, Inc. Following receipt, the frozen plasma samples were immediately stored at -80^{o}C. To generate metabolomic data, a total of 1215 plasma samples from 428 patients at day 0, 413 patients at day 3 and 374 patients at day 7 were analyzed using four ultra-high-performance liquid chromatography/tandem accurate mass spectrometry (UHPLC/MS/MS) methods by Metabolon, Inc. Metabolomic profiling identified 769 metabolites. We reduced baseline noise by removing metabolites with the lowest interquartile range of variability, leaving 578 metabolites. This is a common strategy to reduce baseline noise by removing constant or very weak variables (19, 20). Metabolomic data underwent cube root transformation and Pareto scaling to generate a "normal" distribution (21, 22).

Based on our previous metabolomics analysis of the VITdAL-ICU trial (23), we considered the optimum response to high dose oral vitamin D_3 as an absolute increase in 25(OH)D ≥15 ng/ml from day 0 to day 3. For univariate analysis of day 0, Student's t test was used to determine the significance of each metabolite between vitamin D_3 response groups [25(OH)D < or ≥15 ng/ml from day 0 to day 3] with False Discovery Rate (FDR) adjusted P-values using MetaboAnalyst (24). Day 0 data was 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). The quality of the multivariate model developed was described by R2 and Q2 corresponding to goodness of fit and predictive performance, respectively. Permutation testing was performed to validate the OPLS-DA model (25, 26). Sevenfold cross-validation analysis of variance (CV-ANOVA) was utilized to determine OPLS-DA model significance (26).

For day 0, 3 and 7 repeated measures data, correlations between individual metabolites and absolute increase in 25(OH)D levels from day 0 to day 3 over time were determined utilizing linear mixed effects models correcting for age, Simplified Acute Physiology Score (SAPS) II, admission diagnosis, 25(OH)D at day 0 and plasma day (as the random-intercept). For data visualization purposes, sex-specific separate bipartite graphs (27) were generated in women and men of 198 metabolites previously shown to be significantly changed (increased or decreased) over time with absolute increases in 25(OH)D levels from day 0 to day 3 (23). Rain plots were produced in R-3.6.2 adapted from source code published by Henglin et.al. (28). A multiple test-corrected threshold of $P < 8.65 \times 10^{-5}$ was used to identify all significant associations in the day 0, 3 and 7 repeated measures data (29). All mixed models were analyzed using STATA 14.1MP (College Station, TX).

We evaluated a potential sex-specific mediating effect on the association between the absolute increase in 25(OH)D levels from day 0 to day 3 and individual metabolite abundance, adjusted for age, baseline 25(OH)D, SAPS II and admission diagnosis. Analyses were performed on each of the 578 metabolites at day 3 using the R package mediation (30) to obtain bootstrap P values (N

= 2000 samples) for the mediation effect of sex. Significant mediation was present if the p value was<0.01 and 10% or more of the association was mediated through sex (31, 32).

Results

Baseline characteristics of the analytic cohort were balanced between subjects stratified by absolute increase in 25(OH)D ≥15 ng/ml between day 0 and 3 and then sex for C-reactive protein, SAPS II, body mass index (BMI), diabetes, as well as day 0 levels of VLDL and Triglycerides. Differences existed with respect to age, baseline 25(OH)D levels, intervention status, ICU type as well as day 0 levels of Glucose, Cholesterol, LDL and HDL (Table 1). The overall 28-day mortality of the 428 subject analytic cohort was 22.2%. 28-day mortality differed based on absolute increase in 25(OH)D level between day 0 and day 3 ($\chi 2(1) = 13.3$; $P< 0.001$) but not by gender ($\chi 2(1) = 0.014$; $P= 0.91$).

We next evaluated sex-specific pharmacokinetics of high dose oral vitamin D_3. Though the dose of vitamin D_3 (IU/kg) is higher in women, the pharmacokinetics of 25(OH)D in patients randomized to vitamin D_3 showed similar mean serum 25(OH)D concentrations over time (Supplemental Table 1, Supplemental Figure 1). Compared with women, the pharmacokinetic parameters of 25(OH)D using non-compartmental analysis showed significantly higher normalized AUC0-7 in men ($P < 0.05$) a measure of the extent of drug absorption.

In day 0 plasma samples of female subjects (N=151), no significant differences exist in any individual metabolites by t-test (all FDR-adjusted $P > 0.05$) or in metabolomic profiles by OPLS-DA

(CV-ANOVA p value=0.54) in subjects who respond to vitamin D relative to subjects that do not (Supplemental Table 2). In male subjects at day 0 (N=277), significant differences exist in 21 individual metabolites by t-test (all FDR-adjusted $P < 0.05$) and in metabolomic profiles by OPLS-DA (CV-ANOVA p value 0.009) in those with an increase in 25(OH)D ≥ 15 ng/ml between day 0 and 3 relative to subjects without (Supplemental Table 2). The major findings at day 0 are that men who do not achieve an absolute increase in 25(OH)D ≥15 ng/ml between day 0 and 3 have a significantly lower lysoplasmalogen and plasmalogen abundance at day 0 compared to men with an increase in 25(OH)D ≥15 ng/ml (Supplemental Table 3, Supplemental Figures 2 & 3).

In mixed effects modeling of 1215 total day 0, 3 and 7 plasma samples from the analytic cohort (N=432), 16 metabolites had significantly positive associations in women but not men, dominated by increases in long chain acylcarnitines (Table 2). Eleven metabolites had significantly positive associations in both women and men highlighted by increases in plasmalogens and sphingomyelins. Eight metabolites had significantly positive associations in men but not women. Eighteen metabolites had significantly negative associations in women but not men, dominated by decreases in amino acids. Three metabolites had significantly negative association in both women and men. Sixteen metabolites had significantly negative associations in men but not women, highlighted by decreases in ceramides and fatty acid metabolism (Table 3). The circos plots highlight the overall sex-specific metabolomics differences over time (Figure 1).

Using rain plots to focus on the potentially important mixed-effects modeling results, we visualize more prominent increases in long chain acylcarnitines in women with absolute increase in 25(OH)D level between day 0 and day 3 following intervention (Figures 2 and 3). We also observe more prominent increases in free fatty acids in women with absolute increase in 25(OH)D level between day 0 and day 3 following intervention. The rain plot shows the separation of metabolites that are increased (red) or decreased (blue) in men or women over time with absolute increase in 25(OH)D level between day 0 and day 3 following intervention. Greater significance is shown by increased size of the circles.

We next focused on the potential mediation of the relationship between metabolite abundance and absolute increase in 25(OH)D level between day 0 and day 3 by sex. Mediation analyses in day 3 data revealed no sex-dependent influence on associations between the absolute increase in 25(OH)D and all 578 metabolites (all mediation p values were > 0.01). As both adiposity and critical illness increase free fatty acids (33, 34), we evaluated BMI as a sex-specific mediator of response to vitamin D_3. When individually restricted to female (N=151) or to male subjects (N=277), mediation analyses in day 3 data revealed no influence of BMI on associations between the absolute increase in 25(OH)D and all 578 metabolites (all mediation p values were > 0.01).

Discussion

Our large post-hoc metabolomics study considered temporal changes in the metabolome and 25(OH)D concentration as well as vitamin D_3 dose to determine sex-specific differences in response to vitamin D_3 intervention. Utilization of clinical trial biorepositories and clinical data is

a novel approach to evaluate sex-specific responses which resulted in novel findings. First, our pharmacokinetics data identified sex-specific differences in vitamin D_3 absorption. Second, in our day 0 data, we note sex-specific differences in patients who do or do not mount a response to vitamin D_3. Third, we show a sex-specific metabolomic response to vitamin D_3 associated with differential metabolite patterns over time early in the course of critical illness. Importantly, these patterns manifest as robust increases and decreases in groups of metabolites along the same sub-pathways. All three analyses demonstrate sex-specific metabolism following high dose vitamin D.

The hypercatabolism of critical illness alters how oxidative fuel is produced from amino acids, fatty acids and carbohydrates (35). Stress hormones accentuate lipolysis (33) increasing hydrolyzation of triacylglycerols to release circulating fatty acids and glycerol. At homeostasis, metabolism is sex-specific (36-39). In critical illness, fatty acids and amino acids are the preferred substrate for mitochondrial energy production.

We find that women who respond to high dose vitamin D_3 are different than men (Supplemental Table 3). The major findings at day 0 are that men who achieve an absolute increase in 25(OH)D ≥ 15 ng/ml between day 0 and 3 have a significantly higher lysoplasmalogen and plasmalogen abundance at day 0 compared to men without an increase in 25(OH)D ≥15 ng/ml. Plasmalogens are important antioxidants that act to protect endothelial cells from injury from oxidative stress (40). Plasma lysoplasmalogens are plasmalogen metabolites that are bioactive metabolites that

cause circulating immune cells to adhere to the endothelium (41). Women do not exhibit such differences in plasmalogen and lysoplasmalogen abundance at day 0.

In healthy adults, no sex-specific differences in post-prandial free fatty acid flux exist (42) however, non-oxidative free fatty acid clearance is higher in women (43). We find that critically ill women differ from men in the patterns of change of free fatty acids. Specifically, women have higher and more significant increases in circulating free fatty acids than men (Figure 3). Existing studies may provide some context regarding our observations. White adipose tissue is prominent in regulating metabolic homeostasis (44). The production of the protein hormone Adiponectin by adipocytes is upregulated by vitamin D (45) which lowers hepatic lipogenesis and increases hepatic beta oxidation (44). Sex-specific protein expression in white and brown adipose tissue is demonstrated in animals fed a high fat diet (46, 47). Specifically, protein disulfide isomerase A3 precursor (PDIA3) which up-regulates fatty acid synthase expression (FAS) is increased in females relative to males (46). FAS is essential for fatty acid synthesis. In cell culture models, PDIA3 is found to interact with VDR to rapidly respond to vitamin D (48, 49).

We find that women and men differ in the patterns of change in acylcarnitines. Specifically, women have higher and more significant increases in long chain acylcarnitines than men in response to increased 25(OH)D levels. Experimental and clinical studies may provide some insight into our acylcarnitine observations. Long-chain fatty acids are the main contributor to circulating long-chain acylcarnitines (50, 51). Mitochondria break down fatty acids into ATP via fatty acid β-oxidation (52, 53). Mitochondria in women specifically utilize fatty acids over amino

acids for energy production, create less reactive oxygen species and have an increased oxygen capacity compared to men (54-58).

Circulating acylcarntines are lower in healthy women compared to men (3). Elevations of long-chain acylcarnitines in the circulation result from incomplete mitochondrial fatty acid β-oxidation (50, 59). In exercise and insulin resistance, mitochondrial overload is observed where the abundance of long-chain fatty acids in muscle can be higher than the mitochondrial oxidative capacity resulting in elevated circulating long-chain acylcarnitines (50, 60).

Importantly, FAT/CD36 transporters which facilitate fatty acid entry into mitochondria are more abundant in women (61-63). FAT/CD36 abundance may be important for higher uptake of long-chain fatty acids by mitochondria in women (64). Vitamin D is shown to enhance fatty acid oxidation through upregulation of enzymes and transcription factors including PPAR 1α, pyruvate dehydrogenase kinase 4 as well as carnitine-palmitoyl transferase 1a and 1b (65-67). Our observed increased long-chain acylcarnitines in women relative to men following vitamin D_3 probably reflects an overwhelming of fatty acid β-oxidation by increased fatty acid delivery to mitochondria in women.

There are several strengths to our novel study approach. The sample size is large and the repeated measurement of subjects over time substantially increase our statistical power (68). Linear mixed models are a particularly useful design for determination of metabolite abundance at multiple time points as they account for fixed-effect (age, SAPS II, etc.) and random effect

(sampling day) confounders (69, 70). Further, the rich clinical trial data allows for adjustment of patient variables in the modelling and normalization of metabolite abundance (71). Finally, to adjust for multiple comparisons we employed a conservative Bonferroni corrected P value < 8.65×10^{-5} (29).

Our work has potential limitations inherent to its observational design. Despite our use of samples from a randomized controlled trial and adjustment for multiple confounders, our approach is subject to bias. Though our samples are derived from a randomized controlled trial, our study design is observational, thus causal inference may be limited. The source of samples were patients of Caucasian ethnicity with serum 25(OH)D < 20 ng/ml, thus potentially not generalizable to all critically ill. Further, our study is post-hoc thus should be considered hypothesis generating.

Conclusion

In a large metabolomics study, we demonstrate substantial differences between women and men in the response to high dose vitamin D early in critical illness. Specifically, robust sex-specific differences in pharmacokinetics and metabolomics are present in the response over time to high dose vitamin D. Demonstrating sex-specific differences in the metabolic response to intervention is an important movement towards the understanding of personalized medicine.

Figure Legends

Figure 1. Circos plot of sex-specific changes in metabolites by increase in 25(OH)D following intervention. Bipartite graphs of the associations between absolute increase in 25(OH)D levels from day 0 to day 3 and individual metabolite abundance at day 0, 3 or 7 in women or men. Associations determined utilizing mixed-effects linear regression models in 428 patients correcting for age, Simplified Acute Physiology Score (SAPS) II, admission diagnosis and 25(OH)D at day 0. Panel A. Graph connects the metabolic consequence in women over time to an absolute increase in plasma 25(OH)D with the individual metabolites grouped by metabolite pathway (i.e Lipids) and class (i.e. Acylcarnitines). Panel B. Graph connects the metabolic consequence in men over time to an absolute increase in plasma 25(OH)D with the individual metabolites grouped by metabolite pathway and class. For panel A and B., green curves indicate an increase in the individual metabolite and red curves indicate a decrease in the individual metabolite. The intensity of the color of curves indicates strength of the significance ($-\log_{10}(p)$ value) with the darkest red or darkest green curves having significance at the $-\log_{10}(p) > 4.06$, $p < 8.65 \times 10^{-5}$ level. The more transparent the red or green color, the lower the significance of the change in metabolite. Each bipartite graph has the same structure and order of 198 metabolites presented.

Figure 2 & 3. Rain Plot of metabolites. Correlations between absolute increase in 25(OH)D levels from day 0 to day 3 and individual metabolite abundance at day 0, 3 or 7 in women or men determined utilizing mixed-effects linear regression models correcting for age, Simplified Acute

Physiology Score (SAPS) II, admission diagnosis and 25(OH)D at day 0. 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 red fill color represents an increase in effect size for that metabolite. The blue fill color represents a decrease in effect size for that metabolite. Statistical significance is the multiple test-corrected threshold of $-\log_{10}(p) > 4.06$ which is equivalent to $P < 8.65 \times 10^{-5}$.

Acknowledgements

Conflict of Interest: Dr. Lasky-Su served as a consultant and received personal fees from Metabolon, Inc., outside the submitted work. Dr. Amrein reports receiving lecture fees from Fresenius Kabi. Dr. Dobnig reports receiving lecture fees from Fresenius Kabi. No other financial or other relationships exist that might lead to a conflict of interest.

References

1. Gandhi M, Aweeka F, Greenblatt RM, Blaschke TF. Sex differences in pharmacokinetics and pharmacodynamics. Annu Rev Pharmacol Toxicol. 2004;44:499-523.
2. Kolz M, Johnson T, Sanna S, Teumer A, Vitart V, Perola M, et al. Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. PLoS Genet. 2009;5(6):e1000504.
3. Mittelstrass K, Ried JS, Yu Z, Krumsiek J, Gieger C, Prehn C, et al. Discovery of sexual dimorphisms in metabolic and genetic biomarkers. PLoS Genet. 2011;7(8):e1002215.
4. Dunn WB, Lin W, Broadhurst D, Begley P, Brown M, Zelena E, et al. Molecular phenotyping of a UK population: defining the human serum metabolome. Metabolomics. 2015;11:9-26.
5. Franconi F, Campesi I. Sex Impact on Biomarkers, Pharmacokinetics and Pharmacodynamics. Curr Med Chem. 2017;24(24):2561-75.
6. Kantae V, Krekels EHJ, Esdonk MJV, Lindenburg P, Harms AC, Knibbe CAJ, et al. Integration of pharmacometabolomics with pharmacokinetics and pharmacodynamics: towards personalized drug therapy. Metabolomics. 2017;13(1):9.
7. Karlsson Lind L, von Euler M, Korkmaz S, Schenck-Gustafsson K. Sex differences in drugs: the development of a comprehensive knowledge base to improve gender awareness prescribing. Biol Sex Differ. 2017;8(1):32.
8. Kiehntopf M, Nin N, Bauer M. Metabolism, metabolome, and metabolomics in intensive care: is it time to move beyond monitoring of glucose and lactate? Am J Respir Crit Care Med. 2013;187(9):906-7.
9. Johansson PI, Nakahira K, Rogers AJ, McGeachie MJ, Baron RM, Fredenburgh LE, et al. Plasma mitochondrial DNA and metabolomic alterations in severe critical illness. Crit Care. 2018;22(1):360.
10. Lasky-Su J, Dahlin A, Litonjua AA, Rogers AJ, McGeachie MJ, Baron RM, et al. Metabolome alterations in severe critical illness and vitamin D status. Crit Care. 2017;21(1):193.
11. Amrein K, Schnedl C, Holl A, Riedl R, Christopher KB, Pachler C, et al. Effect of high-dose vitamin D3 on hospital length of stay in critically ill patients with vitamin D deficiency: the VITdAL-ICU randomized clinical trial. JAMA. 2014;312(15):1520-30.
12. Ilahi M, Armas LA, Heaney RP. Pharmacokinetics of a single, large dose of cholecalciferol. The American journal of clinical nutrition. 2008;87(3):688-91.
13. Romagnoli E, Mascia ML, Cipriani C, Fassino V, Mazzei F, D'Erasmo E, et al. Short and long-term variations in serum calciotropic hormones after a single very large dose of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) in the elderly. J Clin Endocrinol Metab. 2008;93(8):3015-20.
14. Cipriani C, Romagnoli E, Scillitani A, Chiodini I, Clerico R, Carnevale V, et al. Effect of a single oral dose of 600,000 IU of cholecalciferol on serum calciotropic hormones in young subjects with vitamin D deficiency: a prospective intervention study. J Clin Endocrinol Metab. 2010;95(10):4771-7.
15. Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D, et al. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA. 2010;303(18):1815-22.

16. Kaddurah-Daouk R, Kristal BS, Weinshilboum RM. Metabolomics: a global biochemical approach to drug response and disease. Annu Rev Pharmacol Toxicol. 2008;48:653-83.
17. Soldin OP, Mattison DR. Sex differences in pharmacokinetics and pharmacodynamics. Clin Pharmacokinet. 2009;48(3):143-57.
18. Seamans KM, Cashman KD. Existing and potentially novel functional markers of vitamin D status: a systematic review. The American journal of clinical nutrition. 2009;89(6):1997S-2008S.
19. Scholz M, Selbig J. Visualization and analysis of molecular data. Methods Mol Biol. 2007;358:87-104.
20. Katajamaa M, Oresic M. Data processing for mass spectrometry-based metabolomics. J Chromatogr A. 2007;1158(1-2):318-28.
21. Struja T, Eckart A, Kutz A, Huber A, Neyer P, Kraenzlin M, et al. Metabolomics for Prediction of Relapse in Graves' Disease: Observational Pilot Study. Front Endocrinol (Lausanne). 2018;9:623.
22. van den Berg RA, Hoefsloot HC, Westerhuis JA, Smilde AK, van der Werf MJ. Centering, scaling, and transformations: improving the biological information content of metabolomics data. BMC Genomics. 2006;7:142.
23. Amrein K, Lasky-Su J, Christopher KB. Metabolomic Basis for Clinical Response to High Dose Vitamin D in Critical Illness. Intensive Care Medicine Experimental. 2018;6(40).
24. Chong J, Xia J. Using MetaboAnalyst 4.0 for Metabolomics Data Analysis, Interpretation, and Integration with Other Omics Data. Methods Mol Biol. 2020;2104:337-60.
25. Westerhuis JA, Hoefsloot HCJ, Smit S, Vis DJ, Smilde AK, van Velzen EJJ, et al. Assessment of PLSDA cross validation. Metabolomics 2008;4(1):81–9.
26. Eriksson L, Trygg J, Wold S. CV-ANOVA for significance testing of PLS and OPLS models. Journal of Chemometrics. 2008;22(11-12):594-600.
27. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, et al. Circos: an information aesthetic for comparative genomics. Genome Res. 2009;19(9):1639-45.
28. Henglin M, Niiranen T, Watrous JD, Lagerborg KA, Antonelli J, Claggett BL, et al. A Single Visualization Technique for Displaying Multiple Metabolite-Phenotype Associations. Metabolites. 2019;9(7).
29. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A. 2003;100(16):9440-5.
30. Dustin T, Yamamoto T, Hirose K, Keele L, Imai K. mediation: R package for causal mediation analysis. Journal of Statistical Software. 2014;59(5):1-38.
31. Masuch A, Pietzner M, Bahls M, Budde K, Kastenmuller G, Zylla S, et al. Metabolomic profiling implicates adiponectin as mediator of a favorable lipoprotein profile associated with NT-proBNP. Cardiovasc Diabetol. 2018;17(1):120.
32. Pietzner M, Budde K, Homuth G, Kastenmuller G, Henning AK, Artati A, et al. Hepatic Steatosis Is Associated With Adverse Molecular Signatures in Subjects Without Diabetes. J Clin Endocrinol Metab. 2018;103(10):3856-68.
33. Fong YM, Marano MA, Moldawer LL, Wei H, Calvano SE, Kenney JS, et al. The acute splanchnic and peripheral tissue metabolic response to endotoxin in humans. J Clin Invest. 1990;85(6):1896-904.
34. Boden G. Obesity and free fatty acids. Endocrinol Metab Clin North Am. 2008;37(3):635-46, viii-ix.

35. Preiser JC, Ichai C, Orban JC, Groeneveld AB. Metabolic response to the stress of critical illness. Br J Anaesth. 2014;113(6):945-54.
36. Murphy MO, Loria AS. Sex-specific effects of stress on metabolic and cardiovascular disease: are women at higher risk? Am J Physiol Regul Integr Comp Physiol. 2017;313(1):R1-R9.
37. Mauvais-Jarvis F. Sex differences in metabolic homeostasis, diabetes, and obesity. Biol Sex Differ. 2015;6:14.
38. Roeters van Lennep JE, Westerveld HT, Erkelens DW, van der Wall EE. Risk factors for coronary heart disease: implications of gender. Cardiovasc Res. 2002;53(3):538-49.
39. Sugiyama MG, Agellon LB. Sex differences in lipid metabolism and metabolic disease risk. Biochem Cell Biol. 2012;90(2):124-41.
40. Wallner S, Schmitz G. Plasmalogens the neglected regulatory and scavenging lipid species. Chem Phys Lipids. 2011;164(6):573-89.
41. White MC, Rastogi P, McHowat J. Lysoplasmenylcholine increases neutrophil adherence to human coronary artery endothelial cells. Am J Physiol Cell Physiol. 2007;293(5):C1467-71.
42. Bickerton AS, Roberts R, Fielding BA, Hodson L, Blaak EE, Wagenmakers AJ, et al. Preferential uptake of dietary Fatty acids in adipose tissue and muscle in the postprandial period. Diabetes. 2007;56(1):168-76.
43. Nielsen S, Guo Z, Albu JB, Klein S, O'Brien PC, Jensen MD. Energy expenditure, sex, and endogenous fuel availability in humans. J Clin Invest. 2003;111(7):981-8.
44. Stern JH, Rutkowski JM, Scherer PE. Adiponectin, Leptin, and Fatty Acids in the Maintenance of Metabolic Homeostasis through Adipose Tissue Crosstalk. Cell Metab. 2016;23(5):770-84.
45. Walker GE, Ricotti R, Roccio M, Moia S, Bellone S, Prodam F, et al. Pediatric obesity and vitamin D deficiency: a proteomic approach identifies multimeric adiponectin as a key link between these conditions. PLoS One. 2014;9(1):e83685.
46. Mukherjee R, Choi JW, Choi DK, Oh TS, Liu H, Yun JW. Gender-dependent protein expression in white adipose tissues of lean and obese rats fed a high fat diet. Cell Physiol Biochem. 2012;29(3-4):617-34.
47. Choi DK, Oh TS, Choi JW, Mukherjee R, Wang X, Liu H, et al. Gender difference in proteome of brown adipose tissues between male and female rats exposed to a high fat diet. Cell Physiol Biochem. 2011;28(5):933-48.
48. Zhang XQ, Pan Y, Yu CH, Xu CF, Xu L, Li YM, et al. PDIA3 Knockdown Exacerbates Free Fatty Acid-Induced Hepatocyte Steatosis and Apoptosis. PLoS One. 2015;10(7):e0133882.
49. Chen J, Doroudi M, Cheung J, Grozier AL, Schwartz Z, Boyan BD. Plasma membrane Pdia3 and VDR interact to elicit rapid responses to 1alpha,25(OH)(2)D(3). Cell Signal. 2013;25(12):2362-73.
50. Koves TR, Ussher JR, Noland RC, Slentz D, Mosedale M, Ilkayeva O, et al. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. Cell Metab. 2008;7(1):45-56.
51. Makrecka-Kuka M, Sevostjanovs E, Vilks K, Volska K, Antone U, Kuka J, et al. Plasma acylcarnitine concentrations reflect the acylcarnitine profile in cardiac tissues. Sci Rep. 2017;7(1):17528.
52. Eaton S. Control of mitochondrial beta-oxidation flux. Prog Lipid Res. 2002;41(3):197-239.

53. Maassen JA, Romijn JA, Heine RJ. Fatty acid-induced mitochondrial uncoupling in adipocytes as a key protective factor against insulin resistance and beta cell dysfunction: a new concept in the pathogenesis of obesity-associated type 2 diabetes mellitus. Diabetologia. 2007;50(10):2036-41.
54. Ventura-Clapier R, Moulin M, Piquereau J, Lemaire C, Mericskay M, Veksler V, et al. Mitochondria: a central target for sex differences in pathologies. Clin Sci (Lond). 2017;131(9):803-22.
55. Malorni W, Campesi I, Straface E, Vella S, Franconi F. Redox features of the cell: a gender perspective. Antioxid Redox Signal. 2007;9(11):1779-801.
56. Vina J, Borras C. Women live longer than men: understanding molecular mechanisms offers opportunities to intervene by using estrogenic compounds. Antioxid Redox Signal. 2010;13(3):269-78.
57. Demarest TG, McCarthy MM. Sex differences in mitochondrial (dys)function: Implications for neuroprotection. J Bioenerg Biomembr. 2015;47(1-2):173-88.
58. Austad SN, Fischer KE. Sex Differences in Lifespan. Cell Metab. 2016;23(6):1022-33.
59. Van Hove JL, Zhang W, Kahler SG, Roe CR, Chen YT, Terada N, et al. Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency: diagnosis by acylcarnitine analysis in blood. Am J Hum Genet. 1993;52(5):958-66.
60. Zhang J, Light AR, Hoppel CL, Campbell C, Chandler CJ, Burnett DJ, et al. Acylcarnitines as markers of exercise-associated fuel partitioning, xenometabolism, and potential signals to muscle afferent neurons. Exp Physiol. 2017;102(1):48-69.
61. Holloway GP, Bezaire V, Heigenhauser GJ, Tandon NN, Glatz JF, Luiken JJ, et al. Mitochondrial long chain fatty acid oxidation, fatty acid translocase/CD36 content and carnitine palmitoyltransferase I activity in human skeletal muscle during aerobic exercise. The Journal of physiology. 2006;571(Pt 1):201-10.
62. Bezaire V, Bruce CR, Heigenhauser GJ, Tandon NN, Glatz JF, Luiken JJ, et al. Identification of fatty acid translocase on human skeletal muscle mitochondrial membranes: essential role in fatty acid oxidation. Am J Physiol Endocrinol Metab. 2006;290(3):E509-15.
63. Kiens B, Roepstorff C, Glatz JF, Bonen A, Schjerling P, Knudsen J, et al. Lipid-binding proteins and lipoprotein lipase activity in human skeletal muscle: influence of physical activity and gender. J Appl Physiol (1985). 2004;97(4):1209-18.
64. Maher AC, Akhtar M, Vockley J, Tarnopolsky MA. Women have higher protein content of beta-oxidation enzymes in skeletal muscle than men. PLoS One. 2010;5(8):e12025.
65. Yin Y, Yu Z, Xia M, Luo X, Lu X, Ling W. Vitamin D attenuates high fat diet-induced hepatic steatosis in rats by modulating lipid metabolism. European journal of clinical investigation. 2012;42(11):1189-96.
66. Marcotorchino J, Tourniaire F, Astier J, Karkeni E, Canault M, Amiot MJ, et al. Vitamin D protects against diet-induced obesity by enhancing fatty acid oxidation. J Nutr Biochem. 2014;25(10):1077-83.
67. Sergeev IN, Song Q. High vitamin D and calcium intakes reduce diet-induced obesity in mice by increasing adipose tissue apoptosis. Molecular nutrition & food research. 2014;58(6):1342-8.
68. Vickers AJ. How many repeated measures in repeated measures designs? Statistical issues for comparative trials. BMC Med Res Methodol. 2003;3:22.

69. Mei Y, Kim BS, Tsui K. Linear mixed effects models for feature selection in high dimensional NMR spectra. Exprt Syst Applic. 2009;36:4703–8.
70. Ernest B, Gooding JR, Campagna SR, Saxton AM, Voy BH. MetabR: an R script for linear model analysis of quantitative metabolomic data. BMC Res Notes. 2012;5:596.
71. Wanichthanarak K, Jeamsripong S, Pornputtapong N, Khoomrung S. Accounting for biological variation with linear mixed-effects modelling improves the quality of clinical metabolomics data. Comput Struct Biotechnol J. 2019;17:611-8.

Table 1. Cohort Characteristics

Characteristic	Absolute increase in 25(OH)D level between day 0 and day 3				Total	P-value
	25(OH)D ≥ 15 ng/ml		25(OH)D < 15 ng/ml			
	Female	Male	Female	Male		
No.	44	73	107	204	428	
Age years Mean (SD)	66.0 (15.4)	62.5 (17.8)	69.1 (12.3)	61.8 (14.4)	64.2 (14.9)	<0.001*
SAPS II Mean (SD)	33.1 (14.3)	33.5 (18.2)	35.2 (15.0)	32.4 (14.8)	33.4 (15.4)	0.14*
CRP µg/mL Day 0 Mean (SD)	126.3 (110.3)	112.5 (86.1)	117.2 (90.5)	133.1 (85.6)	124.9 (89.8)	0.28*
Day 0 25(OH)D ng/ml Mean (SD)	12.9 (4.7)	16.8 (17.7)	13.3 (6.1)	13.5 (4.9)	14.0 (8.8)	<0.001*
Vitamin D_3 Intervention No. (%)	44 (100)	73 (100)	34 (32)	61 (30)	212 (50)	<0.001
Change in 25(OH)D ng/ml Median [IQR]	34.4 (17.6)	32.2 (13.4)	1.8 (4.7)	2.0 (4.6)	10.4 (16.4)	<0.001*
BMI Mean (SD)	27.1 (4.8)	26.8 (4.2)	27.2 (5.7)	27.1 (5.4)	27.1 (5.2)	0.96*
Diabetes History No. (%)	12 (27)	16 (22)	27 (25)	46 (23)	101 (24)	0.87
Glucose Day 0 Mean (SD)	171.0 (70.0)	148.2 (48.5)	154.4 (49.9)	144.3 (47.3)	150.3 (51.4)	0.019*
Cholesterol mg/dl Day 0 Mean (SD)	131.5 (64.5)	126.0 (53.4)	116.0 (48.3)	99.2 (50.1)	111.4 (53.1)	<0.001*
LDL Day 0 Mean (SD)	74.4 (45.6)	74.3 (37.2)	66.0 (34.0)	54.7 (34.4)	62.9 (36.9)	<0.001*
HDL Day 0 Mean (SD)	28.2 (21.4)	23.1 (12.8)	25.1 (17.4)	19.9 (15.3)	22.6 (16.4)	0.0044*
VLDL Day 0 Mean (SD)	25.3 (22.1)	25.8 (17.5)	21.5 (12.4)	21.9 (17.4)	22.8 (16.9)	0.31
Triglycerides Day 0 Mean (SD)	149.2 (124.6)	136.3 (70.7)	139.3 (78.5)	148.8 (106.8)	144.3 (96.8)	0.73
ICU						0.009
Anesthesia ICU No. (%)	5 (11.4)	14 (19.2)	19 (17.8)	45 (22.1)	83 (19.4)	
Cardiac Surgery ICU No. (%)	9 (20.5)	13 (17.8)	33 (30.8)	71 (34.8)	126 (29.4)	
Surgical ICU No. (%)	2 (4.6)	4 (5.5)	5 (4.7)	12 (5.9)	23 (5.4)	
Medicine ICU No. (%)	9 (20.5)	15 (20.6)	22 (20.6)	44 (21.6)	90 (21.0)	
Neurological ICU No. (%)	19 (43.2)	27 (37.0)	28 (26.2)	32 (16.0)	106 (24.8)	
28-day Mortality No. (%)	5 (11)	7 (10)	29 (27)	54 (26)	95 (22)	0.004

Note: Data presented as n (%) unless otherwise indicated. P values determined by chi-square unless designated by (*) then P value determined by ANOVA or ‡ determined by Kruskal-Wallis test.

Table 2. Metabolites increased with response to high dose vitamin D in Women

Metabolite	β Coefficient women	Bonferroni corrected P-value women	-log10(P) women	β Coefficient men	Bonferroni corrected P-value men	-log10(P) men	Super Pathway	Sub Pathway
Homoarginine	0.0074	**3.23 E-06**	**8.25**	0.0053	**9.76 E-03**	**4.77**	Amino Acid	Arginine and Proline Metabolism
Guanidinoacetate	0.006	**2.65 E-03**	**5.34**	0.0024	2.20 E+01	1.42	Amino Acid	Creatine Metabolism
Taurine	0.0051	**8.71 E-03**	**4.82**	0.0035	6.25 E-01	2.97	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
S-methylcysteine	0.0048	**1.83 E-02**	**4.50**	0.0033	**2.67 E-01**	**3.34**	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
Serotonin	0.0034	4.43 E+01	1.12	0.0071	**3.32 E-03**	**5.24**	Amino Acid	Tryptophan Metabolism
Thyroxine	0.0039	7.45 E-02	3.89	0.0064	**6.37 E-07**	**8.96**	Amino Acid	Tyrosine Metabolism
1,5-anhydroglucitol	0.0052	**4.74 E-02**	**4.09**	0.0036	**4.19 E+00**	**2.14**	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism
Carotene diol	0.0066	**2.60 E-04**	**6.35**	0.0068	**2.33 E-06**	**8.39**	Cofactors and Vitamins	Vitamin A Metabolism
Palmitoylcarnitine (C16)	0.0044	**3.10 E-02**	**4.27**	-0.0016	7.78 E+01	0.87	Lipid	Acylcarnitine
Margaroylcarnitine (C17)	0.0054	**7.45 E-03**	**4.89**	-0.0007	3.06 E+02	0.28	Lipid	Acylcarnitine
Stearoylcarnitine (C18)	0.0063	**2.01 E-06**	**8.46**	0.0001	5.65 E+02	0.01	Lipid	Acylcarnitine
dihomo-linolenoylcarnitine (C20:3n3 or 6)	0.0057	**3.05 E-03**	**5.28**	0.0009	2.34 E+02	0.39	Lipid	Acylcarnitine
Lignoceroylcarnitine (C24)	0.0044	**2.25 E-02**	**4.41**	-0.0001	5.14 E+02	0.05	Lipid	Acylcarnitine
Cerotoylcarnitine (C26)	0.0048	**5.56 E-03**	**5.02**	0.0001	5.19 E+02	0.05	Lipid	Acylcarnitine
Behenoyl dihydrosphingomyelin (d18:0/22:0)	0.0051	**1.56 E-02**	**4.57**	0.0034	1.45 E+00	2.60	Lipid	Dihydrosphingomyelins
2-aminoheptanoate	0.0058	**9.78 E-03**	**4.77**	0.0042	1.67 E-01	3.54	Lipid	Fatty Acid, Amino
1-lignoceroyl-GPC (24:0)	0.0049	3.97 E-01	3.16	0.005	**1.70 E-02**	**4.53**	Lipid	Lysophospholipid
1-cerotoyl-GPC (26:0)	0.0023	4.01 E+01	1.16	0.0053	**5.29 E-04**	**6.04**	Lipid	Lysophospholipid
1-(1-enyl-palmitoyl)-GPC (P-16:0)	0.0055	**6.77 E-03**	**4.93**	0.0058	**1.18 E-04**	**6.69**	Lipid	Lysoplasmalogen
1-(1-enyl-stearoyl)-GPE (P-18:0)	0.0046	2.18 E-01	3.42	0.0051	**2.09 E-03**	**5.44**	Lipid	Lysoplasmalogen
1-(1-enyl-oleoyl)-GPE (P-18:1)	0.0037	4.67 E+00	2.09	0.0046	**4.29 E-02**	**4.13**	Lipid	Lysoplasmalogen
1-(1-enyl-palmitoyl)-GPE (P-16:0)	0.0034	5.04 E+00	2.06	0.0044	**2.96 E-02**	**4.29**	Lipid	Lysoplasmalogen
Phosphoethanolamine	0.0055	**4.54 E-02**	**4.11**	0.0034	1.73 E+00	2.53	Lipid	Phospholipid Metabolism
1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4)	0.0074	**1.23 E-07**	**9.67**	0.0057	**2.68 E-05**	**7.33**	Lipid	Plasmalogen
1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (P-16:0/18:2)	0.0059	**1.31 E-03**	**5.65**	0.007	**9.24 E-08**	**9.80**	Lipid	Plasmalogen

Table 2. Metabolites increased with response to high dose vitamin D in Women (continued)

Metabolite	β Coefficient women	Bonferroni corrected P-value women	-log10(P) women	β Coefficient men	Bonferroni corrected P-value men	-log10(P) men	Super Pathway	Sub Pathway
1-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2)	0.0059	**4.43 E-05**	**7.12**	0.0052	**7.81 E-06**	**7.87**	Lipid	Plasmalogen
1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)	0.0051	**4.40 E-04**	**6.12**	0.0046	**8.98 E-04**	**5.81**	Lipid	Plasmalogen
5alpha-pregnan-3beta,20alpha-diol monosulfate	0.0036	1.76 E+01	1.52	0.0048	**2.51 E-02**	**4.36**	Lipid	Progestin Steroids
Isoursodeoxycholate	0.0081	5.22 E-02	4.04	0.0092	**1.59 E-05**	**7.56**	Lipid	Secondary Bile Acid Metabolism
Sphinganin E-1-phosphate	0.0058	**1.23 E-03**	**5.67**	0.0018	3.23 E+01	1.25	Lipid	Sphingolipid Synthesis
Sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)	0.0057	**4.72 E-03**	**5.09**	0.0044	**9.65 E-03**	**4.78**	Lipid	Sphingomyelins
Sphingomyelin (d18:1/19:0, d19:1/18:0)	0.0054	**1.09 E-03**	**5.72**	0.0031	5.45 E-01	3.03	Lipid	Sphingomyelins
Tricosanoyl sphingomyelin (d18:1/23:0)	0.0054	**8.87 E-04**	**5.81**	0.0042	**2.99 E-03**	**5.29**	Lipid	Sphingomyelins
Sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)	0.0054	**3.16 E-03**	**5.26**	0.0037	5.03 E-02	4.06	Lipid	Sphingomyelins
Lignoceroyl sphingomyelin (d18:1/24:0)	0.0044	**5.76 E-03**	**5.00**	0.0039	**1.04 E-02**	**4.75**	Lipid	Sphingomyelins
Uridine	0.0052	**6.24 E-04**	**5.97**	0.0032	1.49 E-01	3.59	Nucleotide	Pyrimidine Metabolism
Gamma-glutamylcitrulline	0.0058	**5.40 E-03**	**5.03**	0.0039	8.10 E-01	2.85	Peptide	Gamma-glutamyl Amino Acid

Note: Significant results presented following mixed effects modeling of each of the 578 individual metabolites measured at day 0, 3 and 7. All estimates adjusted for age, Simplified Acute Physiology Score (SAPS) II, admission diagnosis, 25(OH)D at day 0, 25(OH)D level at day 3 following randomization and plasma day (as the random-intercept). A multiple test-corrected threshold of $p < 8.65 \times 10^{-5}$ = -log10(P) > 4.06 was used to identify all significant associations indicated by bold text. GPC is glycerophosphorylcholine; GPE is glycerophosphoethanolamine.

Table 3. Metabolites decreased with response to high dose vitamin D in Women

Metabolite	β Coefficient women	Bonferroni corrected P-value women	-log10(P) women	β Coefficient men	Bonferroni corrected P-value men	-log10(P) men	Super Pathway	Sub Pathway
Argininate	-0.0052	**3.33 E-02**	**4.24**	-0.0017	8.30 E+01	0.84	Amino Acid	Arginine and Proline Metabolism
N-acetyl-aspartyl-glutamate	-0.0064	**5.26 E-03**	**5.04**	-0.0045	4.86 E-01	3.08	Amino Acid	Glutamate Metabolism
Hydantoin-5-propionic acid	-0.0085	**3.70 E-03**	**5.19**	-0.0048	4.48 E+00	2.11	Amino Acid	Histidine Metabolism
5-(galactosylhydroxy)-L-lysine	-0.006	**1.15 E-02**	**4.70**	-0.0047	1.31 E-01	3.64	Amino Acid	Lysine Metabolism
5-methylthioribose	-0.0062	**2.43 E-02**	**4.38**	-0.0039	3.13 E+00	2.27	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
N-acetyl-isoputreanine	-0.0039	3.27 E+00	2.25	-0.0059	**1.53 E-02**	**4.58**	Amino Acid	Polyamine Metabolism
(N(1) + N(8))-acetylspermidine	-0.0056	**1.68 E-02**	**4.54**	-0.004	8.78 E-01	2.82	Amino Acid	Polyamine Metabolism
N1,N12-diacetylspermine	-0.0081	**3.41 E-03**	**5.23**	-0.0047	1.54 E+00	2.57	Amino Acid	Polyamine Metabolism
Kynurenate	-0.0072	**3.36 E-02**	**4.24**	-0.0052	2.62 E+00	2.34	Amino Acid	Tryptophan Metabolism
N-acetyltryptophan	-0.0171	1.79 E-01	3.51	-0.0201	**1.21 E-03**	**5.68**	Amino Acid	Tryptophan Metabolism
Vanillactate	-0.0063	**1.65 E-02**	**4.54**	-0.0039	2.66 E+01	1.34	Amino Acid	Tyrosine Metabolism
3-methoxytyramine sulfate	-0.0073	**2.46 E-02**	**4.37**	-0.0037	7.35 E+00	1.90	Amino Acid	Tyrosine Metabolism
Homovanillate sulfate	-0.009	**1.42 E-02**	**4.61**	-0.0056	1.05 E+00	2.74	Amino Acid	Tyrosine Metabolism
Glucuronate	-0.0074	**5.45 E-03**	**5.03**	-0.0089	**1.84 E-06**	**8.50**	Carbohydrate	Aminosugar Metabolism
Maltotriose	-0.0074	**3.19 E-02**	**4.26**	-0.0032	1.61 E+01	1.55	Carbohydrate	Glycogen Metabolism
Arabitol	-0.0067	**1.82 E-03**	**5.50**	-0.0046	7.14 E-02	3.91	Carbohydrate	Pentose Metabolism
Gulonate	-0.0072	**1.18 E-02**	**4.69**	-0.0055	1.63 E-01	3.55	Cofactors and Vitamins	Ascorbate and Aldarate Metabolism
Nicotinamide riboside	-0.003	1.33 E+01	1.64	-0.0054	**4.08 E-02**	**4.15**	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism
Pyridoxate	-0.0096	**1.55 E-03**	**5.57**	0.0006	4.22 E+02	0.14	Cofactors and Vitamins	Vitamin B6 Metabolism
2-methylcitrate	-0.0066	**4.24 E-02**	**4.13**	-0.0027	3.49 E+01	1.22	Energy	TCA Cycle
Malonylcarnitine (C3DC)	-0.0039	2.02 E+00	2.46	-0.0045	**2.33 E-02**	**4.40**	Lipid	Acylcarnitine
Octadecenedioylcarnitine (C18:1-DC)	-0.0049	9.88 E-01	2.77	-0.0084	**4.12 E-03**	**5.15**	Lipid	Acylcarnitine
Suberoylcarnitine (C8-DC)	-0.0068	5.54 E-02	4.02	-0.0066	**1.66 E-02**	**4.54**	Lipid	Acylcarnitine
Ceramide (d18:1/17:0, d17:1/18:0)	-0.0006	3.78 E+02	0.18	-0.0047	**2.42 E-02**	**4.38**	Lipid	Ceramides
N-stearoyl-sphingosine (d18:1/18:0)	-0.0021	4.79 E+01	1.08	-0.0048	**1.15 E-02**	**4.70**	Lipid	Ceramides

Table 3. Metabolites decreased with response to high dose vitamin D in Women (continued)

Metabolite	β Coefficient women	Bonferroni corrected P-value women	-log10(P) women	β Coefficient men	Bonferroni corrected P-value men	-log10(P) men	Super Pathway	Sub Pathway
N-stearoyl-sphingadienine (d18:2/18:0)	-0.0023	5.41 E+01	1.03	-0.0051	**2.85 E-03**	**5.31**	Lipid	Ceramides
N-palmitoyl-sphingosine (d18:1/16:0)	-0.0024	2.05 E+01	1.45	-0.0047	**4.43 E-03**	**5.12**	Lipid	Ceramides
Glycochenodeoxycholate sulfate	-0.0068	**3.96 E-02**	**4.16**	-0.0075	1.19 E-01	3.68	Lipid	Diacylglycerol
3-hydroxyadipate	-0.0031	4.47 E+01	1.11	-0.0081	**1.07 E-04**	**6.73**	Lipid	Fatty Acid, Dicarboxylate
3-methyladipate	-0.0051	4.20 E-01	3.14	-0.0065	**1.47 E-02**	**4.60**	Lipid	Fatty Acid, Dicarboxylate
2-hydroxydecanoate	-0.0016	1.48 E+02	0.59	-0.0049	**4.64 E-02**	**4.10**	Lipid	Fatty Acid, Monohydroxy
2-hydroxynervonate	-0.0019	4.36 E+01	1.12	-0.004	**4.99 E-02**	**4.06**	Lipid	Fatty Acid, Monohydroxy
erucate (22:1n9)	-0.0024	3.84 E+01	1.18	-0.0056	**3.03 E-04**	**6.28**	Lipid	Long Chain Fatty Acid
Docosatrienoate (22:3n6)	-0.0011	2.74 E+02	0.32	-0.0076	**9.31 E-05**	**6.79**	Lipid	Polyunsaturated Fatty Acid
1-palmitoyl-2-docosahexaenoyl-GPE (16:0/22:6)	-0.0034	1.92 E+00	2.48	-0.0041	**9.49 E-03**	**4.78**	Lipid	Phosphatidylethanolamine
1-oleoyl-2-linoleoyl-GPE (18:1/18:2)	-0.0058	**1.44 E-03**	**5.60**	-0.0034	1.56 E+00	2.57	Lipid	Phosphatidylethanolamine
1-oleoyl-2-docosahexaenoyl-GPE (18:1/22:6)	-0.006	**2.99 E-03**	**5.29**	-0.006	**9.83 E-05**	**6.77**	Lipid	Phosphatidylethanolamine
Taurocholate	-0.0051	9.20 E+00	1.80	-0.0088	**2.24 E-02**	**4.41**	Lipid	Primary Bile Acid Metabolism
Glycocholate sulfate	-0.0076	1.06 E-01	3.74	-0.0086	**3.56 E-03**	**5.21**	Lipid	Primary Bile Acid Metabolism
N6-carbamoylthreonyladenosine	-0.0062	**2.53 E-03**	**5.36**	-0.004	9.13 E-01	2.80	Nucleotide	Purine Metabolism
N6-succinyladenosine	-0.0068	**1.44 E-03**	**5.60**	-0.0051	1.14 E-01	3.71	Nucleotide	Purine Metabolism
5,6-dihydrouridine	-0.004	5.26 E-01	3.04	-0.0042	**4.90 E-02**	**4.07**	Nucleotide	Pyrimidine Metabolism
N-acetyl-beta-alanine	-0.0055	**1.34 E-03**	**5.63**	-0.0041	**2.39 E-02**	**4.38**	Nucleotide	Pyrimidine Metabolism
Cytidine	-0.0056	**4.14 E-02**	**4.15**	-0.0029	1.05 E+01	1.74	Nucleotide	Pyrimidine Metabolism

Note: Note: Significant results presented following mixed effects modeling of each of the 578 individual metabolites measured at day 0, 3 and 7. All estimates adjusted for age, Simplified Acute Physiology Score (SAPS) II, admission diagnosis, 25(OH)D at day 0, 25(OH)D level at day 3 following randomization and plasma day (as the random-intercept). A multiple test-corrected threshold of $p < 8.65 \times 10^{-5}$ = -log10(P) > 4.06 was used to identify all significant associations indicated by bold text. GPC is glycerophosphorylcholine; GPE is glycerophosphoethanolamine.

Figure 1

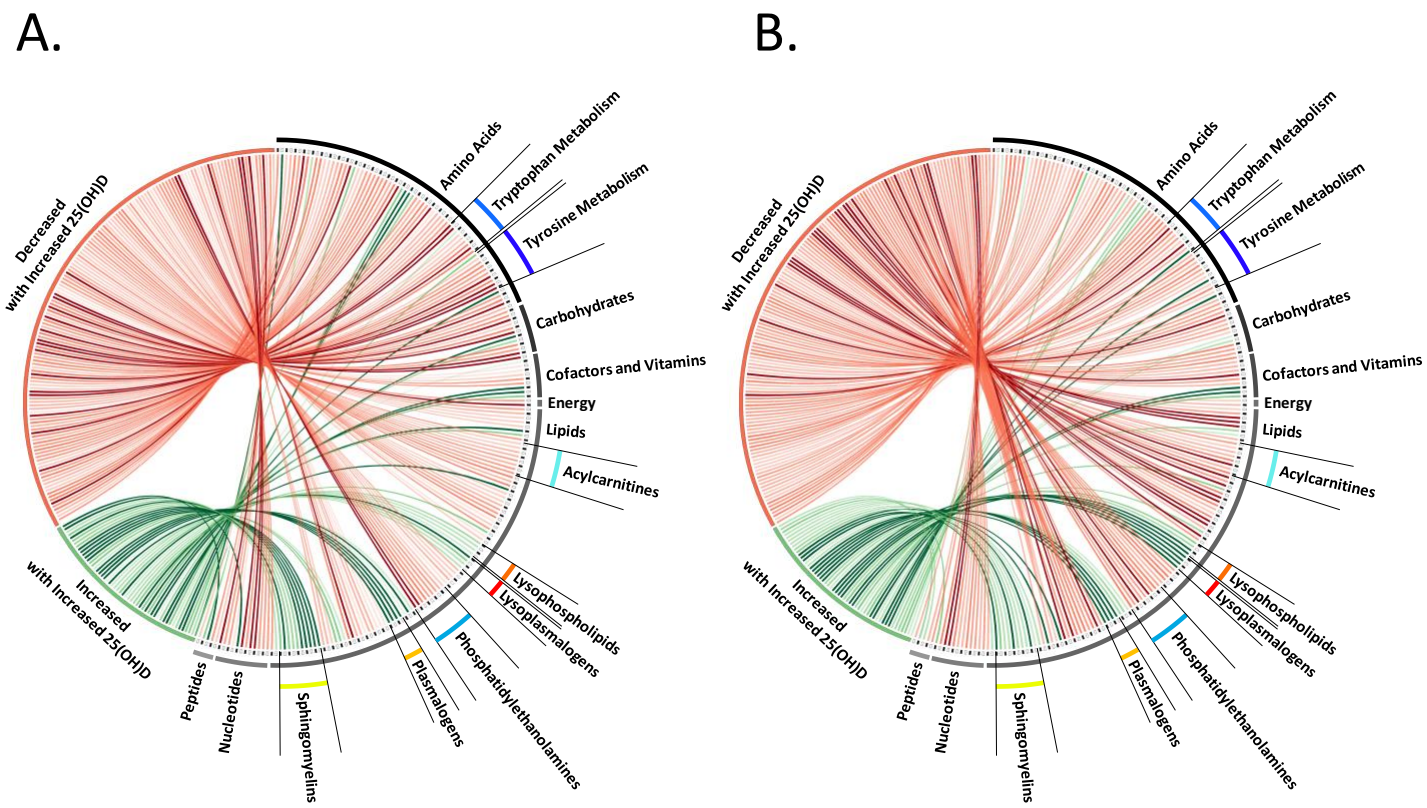

A.
B.
Decreased with Increased 25(OH)D
Increased with Increased 25(OH)D
Amino Acids
Tryptophan Metabolism
Tyrosine Metabolism
Carbohydrates
Cofactors and Vitamins
Energy
Lipids
Acylcarnitines
Lysophospholipids
Lysoplasmalogens
Phosphatidylethanolamines
Plasmalogens
Sphingomyelins
Nucleotides
Peptides

Figure 2. Long Chain Acylcarnitines

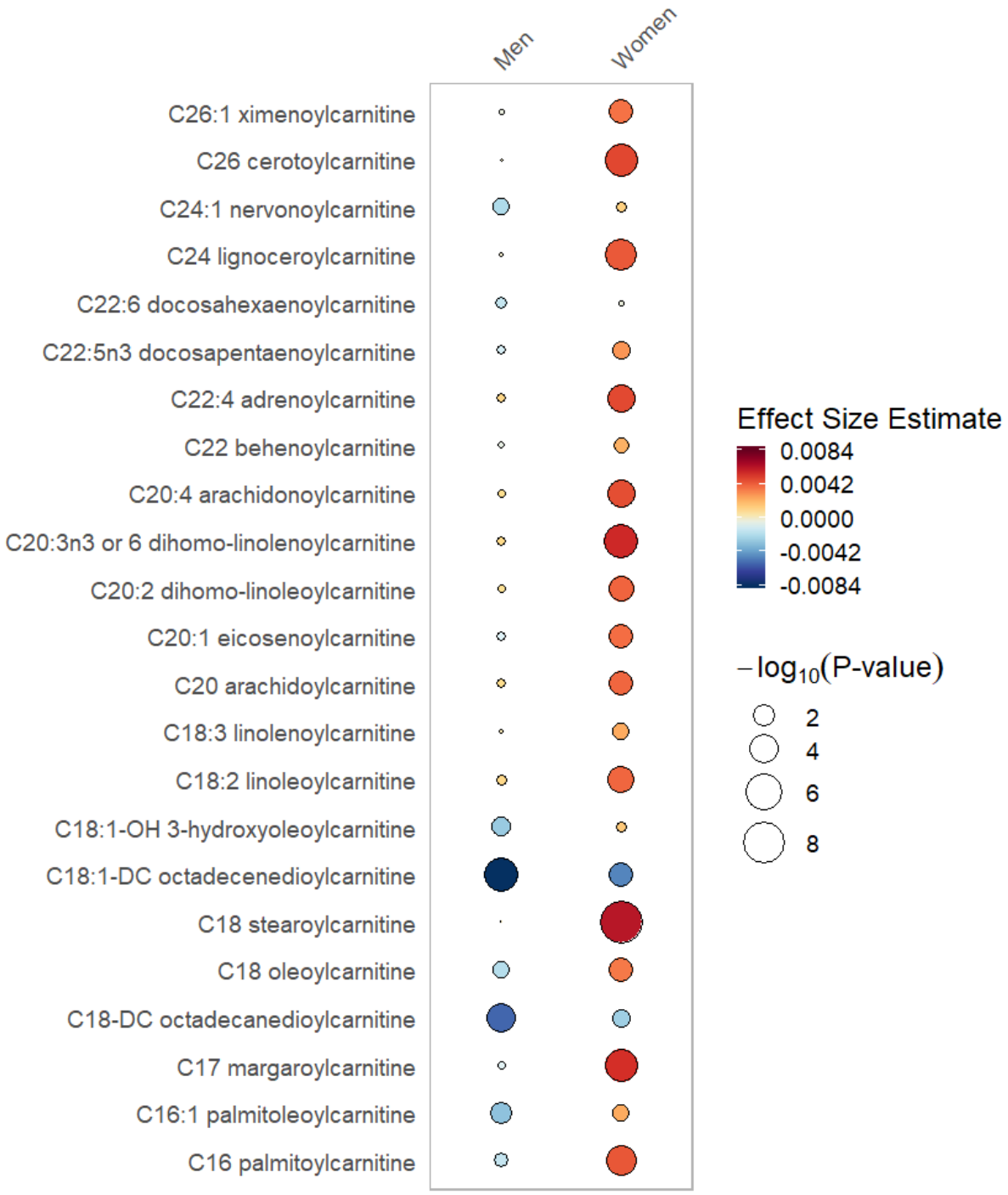
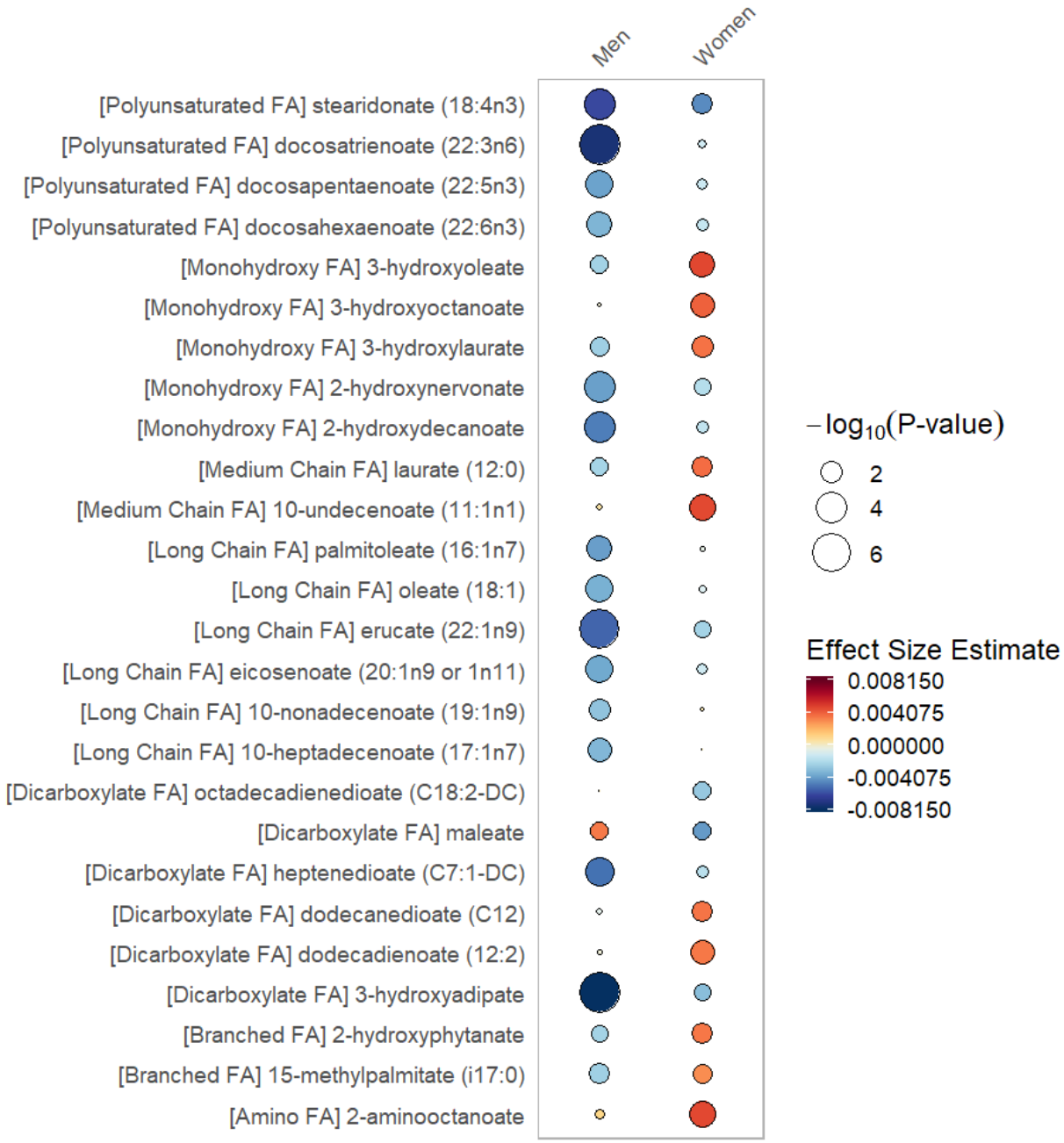


Figure 3. Free Fatty Acids



Supplement

Supplemental Tables

Supplemental Figures

Supplemental Table 1. Pharmacokinetics of High Dose Vitamin D_3

Parameter	Women	Men	P-value
N	67	108	
Dose/kg body weight IU/kg Mean (SD)	7973 (1703)	7788 (1612)	<0.001
C0 ng/ml Mean (SD)	12.47 (4.89)	12.79 (4.50)	0.66
C3 ng/ml Mean (SD)	32.63 (19.63)	33.78 (17.52)	0.69
C7 ng/ml Mean (SD)	35.78 (22.18)	35.50 (20.60)	0.93
AUC0-7 (ng.h/ml)	4850.67 (2714.36)	4965.21 (2453.75)	0.77
AUCnorm (ng.h/ml)/(IU/kg) Median [IQR]	0.57 [0.34, 0.80]	0.67 [0.48, 0.99]	0.034

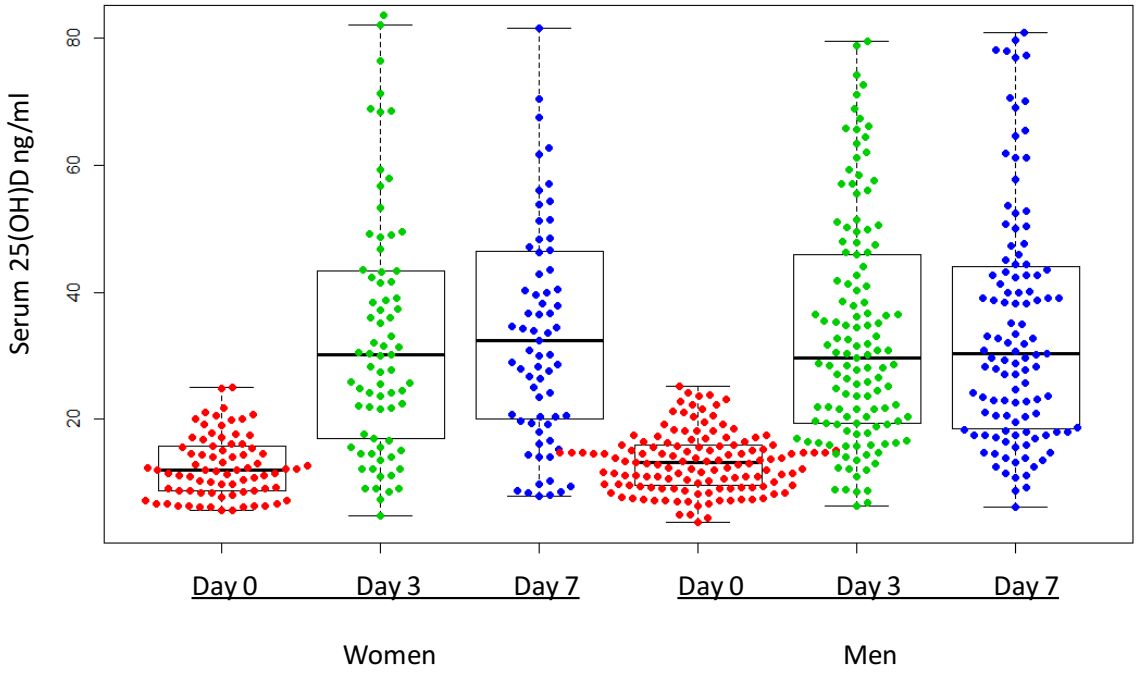
Supplemental Table 2. OPLS-DA model goodness of fit and predictive ability

Characteristic	**OPLS-DA**			**Permutation (*n*=200)**		**CV-ANOVA**
	R2X	R2Y	Q2	R2 intercept (*x*-axis, *y*-axis)	Q2 intercept (*x*-axis, *y*-axis)	P-value
Women	0.141	1.00	0.008	0.00, 0.165	0.00, -0.156	0.54
Men	0.157	1.00	0.034	0.00, 0.097	0.00, -0.107	0.009

Supplemental Table 3. Day 0 Metabolites in Women and Men associated with a subsequent response of 25(OH)D ≥15 ng/ml from day 0 to day 3 following intervention

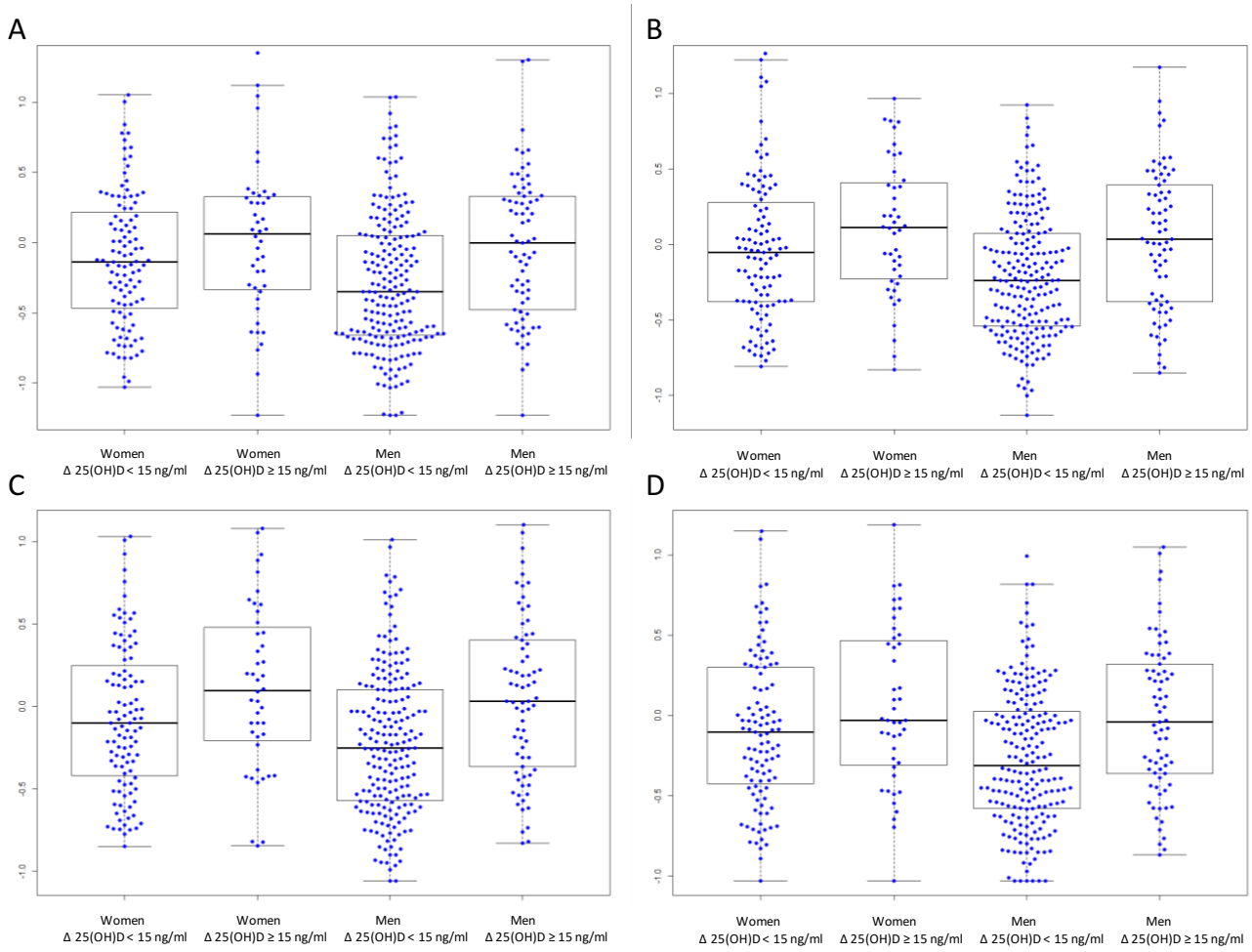
Metabolite	Women		Men		SuperPathway	SubPathway
	t-statistic	FDR adjusted P-value	t-statistic	FDR adjusted P-value		
N-acetylputrescine	-0.86	0.68	-3.21	0.04	Amino Acid	Polyamine Metabolism
Serotonin	0.39	0.85	3.44	0.03	Amino Acid	Tryptophan Metabolism
Thyroxine	1.35	0.49	3.84	0.01	Amino Acid	Tyrosine Metabolism
Gulonate	-2.22	0.34	-3.39	0.03	Cofactors and Vitamins	Ascorbate and Aldarate Metabolism
Bilirubin (E,Z or Z,E)	-1.42	0.48	-3.14	0.05	Cofactors and Vitamins	Hemoglobin and Porphyrin Metabolism
Carotene diol	1.25	0.54	3.18	0.04	Cofactors and Vitamins	Vitamin A Metabolism
Dihomo-linolenoyl-choline	-1.12	0.57	3.24	0.04	Lipid	Fatty Acid Metabolism
Erucate (22:1n9)	-1.43	0.48	-3.57	0.02	Lipid	Long Chain Fatty Acid
2-palmitoyl-GPC (16:0)	0.68	0.73	3.78	0.01	Lipid	Lysophospholipid
1-arachidonoyl-GPC (20:4)	1.63	0.47	3.30	0.04	Lipid	Lysophospholipid
1-(1-enyl-palmitoyl)-GPC (P-16:0)	1.50	0.47	3.69	0.02	Lipid	Lysoplasmalogen
1-(1-enyl-palmitoyl)-GPE (P-16:0)	1.57	0.47	4.02	0.01	Lipid	Lysoplasmalogen
1-(1-enyl-stearoyl)-GPE (P-18:0)	1.91	0.38	4.17	0.01	Lipid	Lysoplasmalogen
1-(1-enyl-oleoyl)-GPE (P-18:1)	1.35	0.49	4.04	0.01	Lipid	Lysoplasmalogen
Glycerophosphorylcholine	1.11	0.57	3.76	0.01	Lipid	Phospholipid
1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4)	2.17	0.34	4.22	0.01	Lipid	Plasmalogen
1-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2)	1.68	0.46	3.91	0.01	Lipid	Plasmalogen
1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (P-16:0/18:2)	1.28	0.52	3.51	0.02	Lipid	Plasmalogen
Lignoceroyl sphingomyelin (d18:1/24:0)	1.10	0.58	3.29	0.04	Lipid	Sphingomyelins
Tricosanoyl sphingomyelin (d18:1/23:0)	1.26	0.54	3.54	0.02	Lipid	Sphingomyelins
N6-succinyladenosine	-3.68	0.06	-3.23	0.04	Nucleotide	Purine Metabolism

Supplemental Figure 1. Day 0, 3 and 7 serum 25(OH)D levels in Women and Men



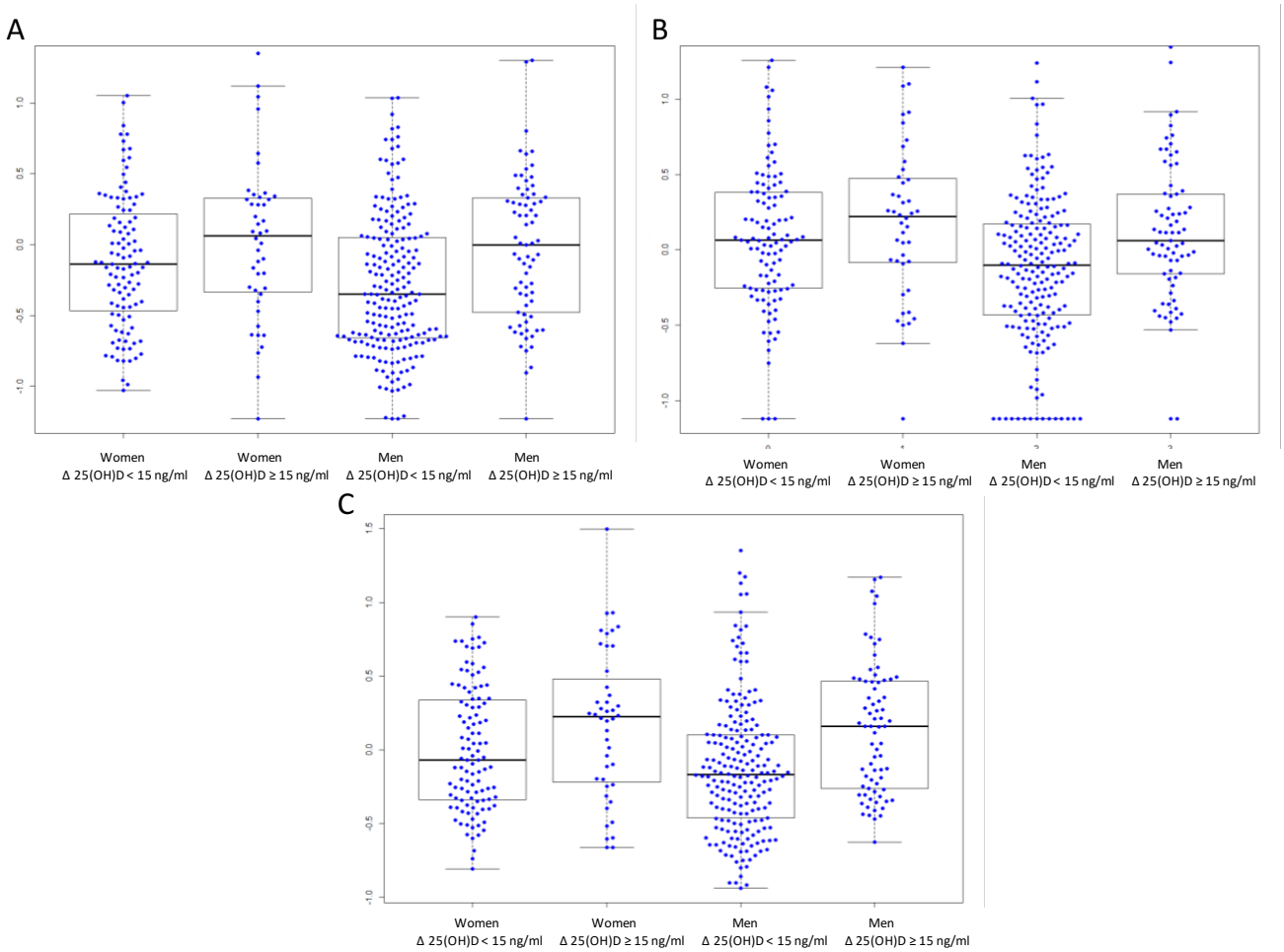
Whisker and box plot overlaid with dot plot showing median and quartile values of the 25(OH)D at day 0, 3 and 7 in women (N=78) and men (N=134) subjects randomized to vitamin D_3.

Supplemental Figure 2. Day 0 Lysoplasmalogens



Whisker and box plot overlaid with dot plot showing median and quartile values of Lysoplasmalogens at day 0 in women and men subjects grouped by subsequent response of 25(OH)D ≥15 ng/ml from day 0 to day 3 following intervention. A: 1-(1-enyl-palmitoyl)-GPC (P-16:0); B: 1-(1-enyl-palmitoyl)-GPE (P-16:0); C: 1-(1-enyl-stearoyl)-GPE (P-18:0); D: 1-(1-enyl-oleoyl)-GPE (P-18:1). GPE is glycerophosphoethanolamine.

Supplemental Figure 3. Day 0 Plasmalogens



Whisker and box plot overlaid with dot plot showing median and quartile values of Plasmalogens at day 0 in women and men subjects grouped by subsequent response of 25(OH)D ≥15 ng/ml from day 0 to day 3 following intervention. A: 1-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2); B: 1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (P-16:0/18:2); C: 1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4). GPE is glycerophosphoethanolamine.

Summary of Conclusions

This body of work describes two metabolomics studies identifying robust sex-specific differences in plasma metabolomics and response to high dose, oral vitamin D_3 in early critical illness.

In the first study, we found that women respond to critical illness differently than men. Single time point data, repeated measures data and metabolite modules showed evidence of sex-specific metabolism. Most prominently, women had increased lysophospolipids and decreased androgenic steroids, bile acid and amino acid metabolism and acylcarnitines relative to men.

In the second study, we found that women respond to high dose, oral vitamin D_3 differently relative to men early in critical illness. Pre-randomization data, mixed methods analysis and pharmacokinetics data showed substantial variations in groups of metabolites along similar sub-pathways and sex-specific differences in drug absorption. Most prominently, an absolute increase in 25(OH)D ≥15 ng/ml between day 0 and 3 in men was associated with a significant increase in lysophospholipids, lysoplasmalogens, and plasmalogens relative to men who did not attain an increase in 25(OH)D ≥15 ng/ml. Although, women did not show evidence of this distinction, they showed significant increases in circulating fatty acids and long chain acylcarnitines relative to men in the response to vitamin D_3.

Identifying these differences in metabolism pathways and in the response to high-dose vitamin D_3, are the first step in not only understanding the delivery of personalized medicine in the critically ill but also in bridging the medical research gender gap.

Discussion and Perspectives

Our novel approach of studying sex-specific metabolomics response to critical illness and the pharmacometabolomic response to high dose vitamin D_3 has particular strengths. We utilized a large number of samples at three time points to determine sex-specific metabolomic patterns. Linear mixed models are especially useful as they account for longitudinal data as well as fixed (i.e. subject characteristics) and random effects (i.e. time) of confounders. We account for multiple comparisons using the Bonferroni adjustment. Finally, we observed known sex-specific differences in metabolism which increases the biological plausibility of our work.

Our work is limited by the inherent limitations of its observational design, and therefore causal inference is limited. Although we used an analytic cohort derived from post-hoc analyses of a randomized controlled trial, and although we adjusted for known covariates, it is possible to have residual confounding due to unmeasured confounders. Further, the studies are post-hoc and subject to bias, as they are asking questions that the trial was not designed to answer.

Vitamin D Receptor activation may regulate the expression of 0.5–5% of the total human genome [1,2]. Vitamin D3 supplementation alters gene expression involved in the immune system, apoptosis, transcription regulation and response to stress [3]. Dose response to vitamin D3 supplementation depends on genetic variation in vitamin D pathway genes that affect the rate of 25-hydroxylation [4-7]. Though cholecalciferol supplementation increases circulating 25(OH)D concentrations proportionally to the dose independent of race [8], human

metabolomics studies are influenced by race [9]. In our studies based on the VITdAL ICU trial, we are unable to study racial differences due to the nature of the Caucasian population studied.

Furthermore, the race of the subjects in the VITdAL ICU trial limits the generalizability of our results to all critically ill adult patients. The VITdAL ICU trial randomized Caucasian, Austrian, critically ill subjects with low vitamin D levels and therefore has an impact on the external validity of our studies. The studies in this body of work were preliminary exploratory analyses. Further studies are needed to not only confirm our findings but to also expand upon the knowledge gap by studying subjects of different races from outside of Europe.

Finally, our use of a large sample size with repeated metabolomics measures in individual patients substantially increases our study power [10]. Due to the high power we were able to use the Bonferroni correction in our analyses to account for the multiple tests being performed. The Bonferroni adjustment is the preferred approach for metabolomics data [11]. We understand that using a conservative approach may limit our ability to find significant associations, however, we also wanted to report metabolites that we have high confidence in their significance. Metabolomics literature is less impactful due to the publication of studies with small sample sizes that are not appropriately adjusted for multiple comparisons. Using the Bonferroni method increases the likelihood of a type II error, however in metabolomics studies, not accounting for the multiple tests performed can increase the likelihood of a type I error.

The sex-specific plasma metabolite modules and response to intervention identified in our analyses shed light on the variations in the response to critical illness stressors between men and women. The observed highly significant differences in multiple metabolites of the same sub-pathways, highlight the need for tailored therapy with regards to drug dosing and adverse event monitoring. Although subsequent studies are needed to confirm the exploratory analyses, our findings show the importance of increasing enrollment of women subjects in clinical research. Bridging the medical research gender gap is not only equitable but is also necessary for understanding personalized healthcare delivery.

References

1. Tuoresmaki P, Vaisanen S, Neme A, Heikkinen S, Carlberg C. Patterns of genome-wide VDR locations. PLoS One. 2014;9(4):e96105.

2. Ramagopalan SV, Heger A, Berlanga AJ, et al. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. Genome Res. 2010;20(10):1352-1360.

3. Hossein-nezhad A, Spira A, Holick MF. Influence of vitamin D status and vitamin D3 supplementation on genome wide expression of white blood cells: a randomized double-blind clinical trial. PLoS One. 2013;8(3):e58725.

4. Fu L, Yun F, Oczak M, Wong BY, Vieth R, Cole DE. Common genetic variants of the vitamin D binding protein (DBP) predict differences in response of serum 25-hydroxyvitamin D [25(OH)D] to vitamin D supplementation. Clin Biochem. 2009;42(10-11):1174-1177.

5. Nimitphong H, Saetung S, Chanprasertyotin S, Chailurkit LO, Ongphiphadhanakul B. Changes in circulating 25-hydroxyvitamin D according to vitamin D binding protein genotypes after vitamin D(3) or D(2)supplementation. Nutr J. 2013;12:39.

6. Waterhouse M, Tran B, Armstrong BK, et al. Environmental, personal, and genetic determinants of response to vitamin D supplementation in older adults. J Clin Endocrinol Metab. 2014;99(7):E1332-1340.

7. Zhang M, Zhao LJ, Zhou Y, et al. SNP rs11185644 of RXRA gene is identified for dose-response variability to vitamin D3 supplementation: a randomized clinical trial. Sci Rep. 2017;7:40593.

8. Alzaman NS, Dawson-Hughes B, Nelson J, D'Alessio D, Pittas AG. Vitamin D status of black and white Americans and changes in vitamin D metabolites after varied doses of vitamin D supplementation. The American journal of clinical nutrition. 2016;104(1):205-214.

9. Dunn WB, Lin W, Broadhurst D, et al. Molecular phenotyping of a UK population: defining the human serum metabolome. Metabolomics. 2015;11:9-26.

10. Fang H, Brooks GP, Rizzo ML, Espy KA, Barcikowski RS. A Monte Carlo Power Analysis of Traditional Repeated Measures and Hierarchical Multivariate Linear Models in Longitudinal Data Analysis. J Mod Appl Stat Methods. 2008;7(1).

11. Mutter S, Worden C, Paxton K, Makinen VP. Statistical reporting of metabolomics data: experience from a high-throughput NMR platform and epidemiological applications. Metabolomics. 2019;16(1):5.