AWARD NUMBER W81XWH-11-1-0451

TITLE: Metabolomic Profiling of Prostate Cancer Progression During Active Surveillance

PRINCIPAL INVESTIGATOR: Bruce J. Trock, Ph.D.

CONTRACTING ORGANIZATION: Johns Hopkins School of Medicine
Baltimore, MD 21287

REPORT DATE: January 2018

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
January 2018	FINAL	30Sep2011 - 29Oct2017

4. TITLE AND SUBTITLE

Metabolomic profiling of prostate cancer progression during active surveillance

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0451

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Bruce J. Trock, Ph.D.

E-Mail: btrock@jhmi.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Johns Hopkins School of Medicine
Baltimore, MD 21287

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
This final report covers activities during the entire project period and including the no cost extension period, the completion of laboratory analyses and most statistical analyses. Delays in the project resulted in some changes to project aims; delays were due to personnel changes and loss of key personnel due to illness, difficulty obtaining appropriate samples from Pathology Department, and lengthy evaluation of HRPO issues. However, the overall objectives were met, with requisite samples obtained from 100 active surveillance patients who had biopsy upgrading within 3 years, and 100 who were free of upgrading for $\geq$5 years. 850 named metabolites were identified in the serum samples, with 169 significantly elevated or decreased in cases compared to controls. In urine there were 691 named metabolites, with 169 significantly elevated or decreased in cases compared to controls. Because of the time element inherent in the case and control definition, there were differences in sample age between cases and controls, creating a potential bias. Analyses were restricted to metabolites that were not correlated with sample age, excluding those with a p-value for correlation $\leq$0.10, resulting in 637 serum metabolites and 545 urine metabolites remaining in the analysis pipeline. Dimension reduction was achieved by excluding metabolites with low expression, and those with mean fold-difference between the 25th & 75th percentiles, and non-significant univariate Wilcoxon rank sum test. Regularized logistic regression analysis with an elastic net penalty was applied to the reduced metabolite sets, identifying 14 serum metabolites and 9 urine metabolites significantly associated with Gleason score upgrading, with AUCs of 0.793 and 0.711, respectively. There was little overlap between the metabolites identified in both sample matrices. Analyses still to be completed will seek to refine these models, then incorporate them into signatures that include established prognostic factors for upgrading in active surveillance. Additional evaluation will consider the biological pathways and mechanisms associated with metabolites in the signatures, and potential associations of metabolites with dietary and lifestyle factors.

15. SUBJECT TERMS
Prostate cancer, active surveillance, Gleason grade, metabolomics

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	62	19b. TELEPHONE NUMBER *(include area code)*

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PI – Signature

Date: September 10, 2018

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INTRODUCTION

This is the Final Report for this project. It provides a summary of activities from 9/30/11 – 10/29/17, including the no cost extension (NCE) period following the end of the original period of performance of the grant. The current report describes the following:

Year 1: initial activities, enrollment, personnel problems, changes to increase accrual, pilot study
Year 2: progress and problems with personnel and changes in scientific knowledge, modification of Aim 1 (no change to Aim 2)
Year 3: progress, change in sample source for Aim 1, pilot study to evaluate whether PCA3 buffer in urine tubes for Aim 2 affects metabolite assay sensitivity, need for a NCE
Year 4: HRPO issues, study halt, resolution and resumption of study, samples shipped to industry partner (Metabolon, Inc.)
Year 5: Analyses performed by Metabolon, results of metabolomics analysis, initial statistical analysis methods and results, plans for finalizing analyses and submitting a manuscript, tasks and subtasks from SOW that were completed

Note: Although results of pilot metabolomics are described with respect to Years 2 and 3, to avoid repetition we will only describe the assay protocol and methods in detail when describing the main study results in Year 5.

BODY

YEAR 1

The original study objectives and specific aims are as follows:

Aim 1. Develop distinct metabolomic profiles to discriminate pure Gleason 6 tumors (without grade 4) from pure Gleason 7 (3+4 or 4+3) tumors in frozen tissue from men undergoing prostatectomy, and determine whether the profile can be detected in matched urine or serum.

Aim 2. Determine whether the metabolomic profile developed in Aim 1, when measured in baseline urine or serum samples from active surveillance (AS) men, can distinguish those who are upgraded from Gleason 6 to Gleason 7 within 3 years ("cases") vs. those who are not upgraded for ≥5 years ("controls"). Also, correlate *changes* in urine or serum metabolomic profiles from baseline to follow-up samples in AS men who do and do not progress.

Progress

Initial Activities. An enrollment tracking database was developed using Excel, and the Material Transfer Agreement was entered into with Metabolon, Inc. Process of prospectively enrolling patients scheduled for prostatectomy (RP) was established, as follows. The Research Nurse identifies men scheduled for RP (HIPAA Waiver of Authorization approved for Research Nurse to review the surgery schedule), mails eligible men a packet describing the study and including a consent form for them to sign and mail back in a postage-paid envelope. When the consent form is received in the mail, the Research Nurse checks the biopsy log maintained by the Pathology department to determine if the biopsies meet study eligibility criteria: biopsy contains only Gleason score 6 or only Gleason score 7. If the consented patient meets eligibility criteria, the Pathology Tech is notified to harvest the specimen on the date of surgery (if it meets harvesting requirements, below). If the consent form is not received by 1 week before surgery, the Research Nurse calls the participant and asks them to bring the consent form with them when they come for pre-operative blood testing (day before surgery), when she will arrange to collect the form. Research blood is collected at the same time as pre-op blood testing, and a urine sample is also collected by the Research Nurse.

RP tissue harvesting requirements: RP specimens selected for harvesting of frozen tissue must weigh >25 grams, and also meet one of the following criteria:

a. Diagnostic biopsy specimens have at least 3 cores containing cancer
b. At least one diagnostic biopsy core has ≥50% of the core occupied by tumor.
c. Gleason pattern 4 or 5 in the biopsy

Four punch research biopsies (7 mm diameter) are taken from a palpable nodule if present in the selected RP specimen, or if there is no palpable nodule, punch biopsies are taken from the area in the prostate where the biopsy report indicated the presence of cancer. Because of the predominance of small tumors scheduled for RP during this time period, **only 15-20% of RPs at Johns Hopkins are selected for harvesting frozen tissue**. The number harvested is also reduced by our biopsy criteria that requires only Gleason 6 or only Gleason 7 in the diagnostic biopsy cores. **Additionally, when H&Es are taken from the punch biopsy core, only about 65% contain cancer**.

These stringent requirements and realities of our RP specimens resulted in enrollment being slower than anticipated. By the end of Year 1 481 RP cases were reviewed, 112 met eligibility criteria, 55 were consented, and only 31 patients were enrolled with adequate samples collected; less than would be needed to reach the Aim 1 goal of 50 Gleason 6 and 50 Gleason 7 tumors within 3 years.

Personnel Issues, Other Barriers to Enrollment, Proposed Modifications. The Pathology Fellow who was to oversee tissue harvesting left Johns Hopkins to return to his home country. The process of recruiting a new Pathology Fellow took some time, and this process was

controlled by the Pathology Department, not this project. Furthermore, overall frozen tissue harvesting performed by the Pathology Department was also slower than normal because 2 of the technicians who assist in the general harvesting effort (positions not funded by this grant) went on maternity leave during Year 1. In addition, the study Research Nurse is dealing with a serious chronic disease and had to take more sick leave than usual, which reduced the number of eligible patients who could be consented.

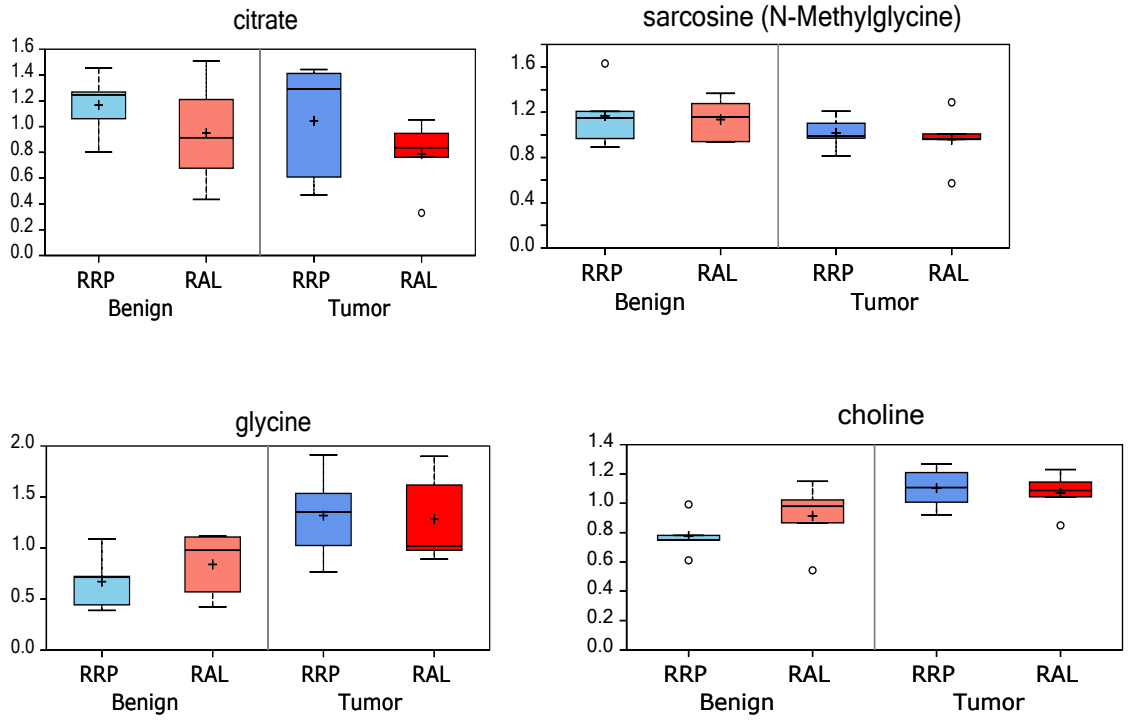
44% of cases that were not selected for harvesting were due either to the biopsy core containing both Gleason 6 and 7, or the patient having a previous cancer or transurethral resection of the prostate (TURP) for BPH, and additional 5% were lost because the consent form arrived too late. We decided to drop the requirement for pure Gleason 7 in the core (i.e. not additional foci of Gleason 6) because the biology is driven by the Gleason pattern of individual glands, and we dissect Gleason pattern 3 and pattern 4 separately in the punch cores, allowing comparison of metabolomics of Gleason pattern 3 vs. 4. We also decided to allow men with previous cancer, as long as no systemic treatment was received during the previous 6 months, and disease was not progressive.

To decrease the number of patients whose consent arrives too late, we decided to call patients 1 week after sending the packet (rather than 2) to check if they received it and have questions.

Pilot Study.

In February 2012 we sent matched tumor and benign tissue samples from 5 open prostatectomy (RRP) cases and 5 robotic assisted laparoscopic prostatectomy (RAL) cases (including 3 Gleason 6 and 7 Gleason 7 cases) to our collaborators at Metabolomics to ensure that their laboratory procedures worked well with our samples and to optimize the protocol. In particular, we wanted to determine whether samples obtained from RRP cases differed from RAL cases; the latter now comprise the majority of prostatectomies performed here.

Data were received in March 2012. A total of 405 biochemicals were identified in this data set (304 named biochemicals + 101 structurally unnamed biochemicals). There were no apparent differences between specimens obtained by RRP vs. RAL; this was true for tumor as well as normal. Given the large number of metabolitess measured and the small sample size it was expected that some differences would be detected purely by chance. The number of specimens is too small for inference, and the pilot sample set was assembled only to ensure that adequate signal was obtained and to look for consistent differences between RRP and RAL, which were not observed. Below are box plots showing comparison of RRP vs. RAL, in tumor and normal, for a few exemplar metabolites. These results demonstrated that the metabolomics analysis performed by our collaborator Metabolon was feasible with our samples, and suggested that differences in surgical technique were unlikely to induce artifact.



YEAR 1 CONCLUSIONS

During the first year of this project we encountered significant difficulties in reaching accrual goals. Some of these are system-related and cannot be improved upon. However, it is anticipated that the changes we implemented would reduce the losses to accrual by approximately 50%.

A pilot study demonstrated that our samples were adequate for metabolomics analyses, and there were no obvious differences in samples from open vs. robot-assisted surgery

YEAR 2

Progress

Change in Aim 1. Our original plan was to develop metabolomic profiles from Gleason 6 and Gleason 7 prostatectomy tissue, then evaluate the prognostic value of the profiles in matched urine and serum samples. However, in the time since the grant proposal was written, our

industry collaborator (Metabolon, Inc.) **identified a metabolomic profile from prostatectomy tissue that was associated with aggressive prostate cancer**.[1] Thus, we revised Aim 1 to validate the McDunn profile in urine and serum from prostatectomy patients with Gleason 6 vs. 7 tumors. Aim 2 did not change, but would evaluate either on the McDunn signature, or if a modified signature is developed in Aim 1.

Personnel. Shortly after we submitted the Year 1 progress report the Research Nurse for the study, Patricia Kolmer, who was unfortunately suffering from a serious chronic disease passed away in May 2013. With her loss we were unable to rely on prospective enrollment being sufficient to complete the study and we began identifying patients with frozen prostatectomy tissue and matched urine and serum already available in the Prostate Cancer Biorepository Network (PCBN), a DOD-funded biorepository with Dr. Trock as PI.

We also decided to begin assembling the urine and serum samples from the AS patients for Aim 2 because samples had already been collected from over 1200 JHU AS patients. We identified 150 patients who were upgraded (Gleason 7 or higher) at an annual surveillance biopsy, and 100 patients who had been followed for at least 5 years without an unfavorable biopsy (i.e. all annual surveillance biopsies have been Gleason 6, with no more than 2 cores positive for cancer and no more than 50% of any biopsy core involved with tumor).

YEAR 3

Progress

Samples for Aims 1 & 2. With the shift in focus to collection of paired urine and serum samples from men with Gleason 6 or Gleason 7 tumors at prostatectomy we began to obtain samples from a biorepository of existing, prospectively collected specimens developed and maintained by Dr. Alan Partin ("Development and Evaluation of a Tumor Marker for Prostate Cancer," IRB protocol #NA00047205). However, we were only able to identify 80 patients who met our criteria and had matched serum and urine, and an additional 6 patients who had only urine samples available. There was no other readily available source of matched serum and urine samples from prostatectomy patients. However, under a previous DOD-funded study "Molecular Epidemiology of Prostate Cancer" (PI: Dr. Trock, WIRB protocol #20011642), serum samples from JHU prostatectomy patients had been collected and stored. Although that award had ended, Dr. Trock maintained the protocol and enrollment of patients and sample collection using institutional funds. From those existing samples we identified 21 patients whose serum samples had been collected during a similar time period as the samples from Dr. Partin's biorepository. The samples from both Dr. Partin's and Dr. Trock's biorepositories, totaling 80 patients with matched serum and urine, 21 patients with serum, and 6 patients with urine (total 187 samples) were sent to Metabolon, Inc. for analysis to accomplish Aim 1.

Aim 2 relies on urine or serum samples (whichever performs better in Aim 1) from 50 men in the AS program who have not experienced biopsy reclassification for at least 5 years and 50 men who had biopsy upgrading (from Gleason 6 to Gleason 7 or higher) within 5 years. The urine and serum samples were prospectively collected under the protocol "Active Surveillance for Prostate Cancer" (PI: H. Ballentine Carter, IRB protocol #NA00045103). However, the urine samples were stored in PCA3 buffer (Hologic Gen-Probe, Inc.), whereas Metabolon's urine metabolomics assay was optimized on unbuffered urine, possibly affecting assay sensitivity. Accordingly, urine specimens were collected from 25 men in the AS program, and each sample was divided into 2 aliquots, half of which was stored in PCA3 buffer, and half stored without buffer, along with a sample of buffer solution. These samples were sent to Metabolon for analysis.

A total of 582 componds of known identity ("named biochemicals") were identified, and an additional 515 compounds of unknown structural identify ("unnamed biochemicals"). The primary result relevant to the current study is that the majority of compounds were detected at reduced levels in the buffered samples. However, of the 582 named biochemicals, only 32 (5.5%) were not detectable at all in a majority of buffered urine samples. Thus, for the purposes of the current study, the *relative levels* in Gleason 6 vs. Gleason 7 tumors should still be informative, even if absolute levels are somewhat reduced.

Based on these results we identified 100 men from the AS program who have not experienced biopsy reclassification, and 100 men who experienced biopsy upgrading within 5 years, and who have available serum and urine samples. Aim 1 samples sent to Metabolon would determine whether urine or serum analyses performed better at distinguishing Gleason 6 from 7, whereupon the appropriate samples would be retrieved and aliquotted and sent to Metabolon for analysis of Aim 2.

Key Research Accomplishments

Demonstration that the majority of metabolites and biochemical compounds that can be detected in raw (unbuffered) urine can also be detected in buffered urine at sensitivity sufficient for relative comparisons between Gleason 6 vs. Gleason 7 prostate cancer patients.

No Cost Extension Request

In a letter to Joshua McKean dated 8/27/14, we detailed problems described above (Years 1 & 2) that led to the delay of the study, the need to modify Aim 1 to focus only on serum and urine, and that we had identified patients with samples who met our criteria. Due to the delays described

above, we had not spent the award funds that were budgeted to pay Metabolon for the assays (fee for service) and the funds were returned to the DOD. The NCE was requested to restore the funds so that we could authorize Metabolon to perform the analyses.

YEAR 4

Progress

NCE, HRPO and IRB issues: On April 24, 2014, Karen Eaton, from USAMRMC ORP HRPO, emailed Dr. Trock requesting clarification of IRB status for the project, because the project proposed to use samples from other, IRB-approved protocols led by other Principal Investigators (but on which Dr. Trock was an investigator). Dr. Trock ultimately sent Ms. Eaton a detailed response with requested documents on May 19, 2015 (submitted as **Appendix 2** in the Year 4 Progress Report). Based on the information that Dr. Trock sent to Ms. Eaton she determined that Dr. Trock needed to submit a separate application to the Johns Hopkins School of Medicine (JHM) IRB. Because of this determination Dr. Trock was requested to halt study-related activities until the project with Dr. Trock as PI received IRB approval. Dr. Trock halted the study and submitted a protocol to the JHM IRB (submitted as **Appendix 3** in the Year 4 Progress Report). This put approval of the NCE (described above for Year 3) on hold until the IRB issues had been resolved to HRPO's satisfaction.

The protocol with Dr. Trock as the PI was approved by the JHM IRB on July 12, 2016 (approval letter submitted as **Appendix 4** in the Year 4 Progress Report). HRPO issued approval of the protocol through an email from Nancy E. Englar, MHL, BSN, RN, CIP on July 27, 2016. The NCE was approved by USAMRAA on August 24, 2016 as indicated in an email from Michelle L. Cromwell on that date. The original period of performance was extended to October 29, 2017

At the time the study was halted, samples for Aim 1 had already been sent to Metabolon, Inc. (without any HIPAA-defined PHI). These samples comprised 80 patients with matched serum and urine, 21 patients with serum only, and 6 patients with urine only (total 187 samples). A series of discussions ensued with scientists at Metabolon, Inc. about the most rigorous approach to analyzing the samples. The issues were as follows. The samples came from 2 different cohorts: a study conducted by Dr. Partin that collected both serum and urine, and a study conducted previously by Dr. Trock that collected only serum (these studies described in 3rd Annual Report).

The Metabolon collaborators felt that potential differences in the Partin and Trock cohorts, and lack of both serum and urine for all patients could introduce batch effects and unwanted variability. This would be exacerbated by the fact that Aim 2 patients came from a 3rd cohort. **A decision was made to not analyze the Aim 1 patients that were stored in freezers at**

Metabolon, and to perform discovery using both serum and urine from the Aim 2 patients. This would provide a stronger discovery platform and keep discovery in the clinical context that was the ultimate goal of the project, i.e. clinical decision-making in active surveillance. The Aim 1 samples could be stored until it was determined whether they could be useful for further analyses, or ultimately returned to Dr. Trock.

Serum and urine samples from 100 patients in each of the Aim 2 groups previously identified from the JHU AS IRB-approved database (described above for Year 3) were aliquotted and shipped to Metabolon for analysis in 2 batches on Feb 22 and Feb 27, 2017.

YEAR 5

Progress

Metabolomic analysis of the Aim 2 serum and urine samples and preliminary data analyses performed by Metabolon were completed in April 2017. The methodology for the metabolomic assays, preliminary data analyses conducted by Metabolon, and data analyses conducted by Dr. Trock will be described, and the results presented.

Metabolomic assay methods.

Similar methods were used for serum and urine, except where noted otherwise, as follows.

Sample Accessioning: Following receipt, samples were inventoried and immediately stored at -80oC. Each sample received was accessioned with a unique identifier into the Metabolon LIMS system, all portions of any sample were automatically assigned their own unique identifiers by the LIMS when a new task was created; the relationship of these samples was also tracked. All samples were maintained at -80oC until processed
.
Sample Preparation: Samples were prepared using the automated MicroLab STAR® system from Hamilton Company. Several recovery standards were added prior to the first step in the extraction process for QC purposes. To remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix, and to recover chemically diverse metabolites, proteins were precipitated with methanol under vigorous shaking for 2 min (Glen Mills GenoGrinder 2000) followed by centrifugation. Both urine and serum samples were extracted on an equal volume basis. The resulting extract was divided into five fractions: two for analysis by two separate reverse phase (RP)/UPLC-MS/MS methods with positive ion mode electrospray ionization (ESI), one for analysis by RP/UPLC-MS/MS with negative ion mode ESI, one for analysis by HILIC/UPLC-MS/MS with negative ion mode ESI, and one sample was

reserved for backup. Samples were placed briefly on a TurboVap® (Zymark) to remove the organic solvent. The sample extracts were stored overnight under nitrogen before preparation for analysis.

QA/QC: Several types of controls were analyzed in concert with the experimental samples: a pooled matrix sample generated by taking a small volume of each experimental sample (or alternatively, use of a pool of well-characterized human plasma) served as a technical replicate throughout the data set; extracted water samples served as process blanks; and a cocktail of QC standards that were carefully chosen not to interfere with the measurement of endogenous compounds were spiked into every analyzed sample, allowed instrument performance monitoring and aided chromatographic alignment. Instrument variability was determined by calculating the median relative standard deviation (RSD) for the standards that were added to each sample prior to injection into the mass spectrometers. Overall process variability was determined by calculating the median RSD for all endogenous metabolites (i.e., non-instrument standards) present in 100% of the pooled matrix samples. Experimental samples were randomized across the platform run with QC samples spaced evenly among the injections,

Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS): All methods utilized a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. The sample extract was dried then reconstituted in solvents. Each reconstitution solvent contained a series of standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot was analyzed using acidic positive ion conditions, chromatographically optimized for more hydrophilic compounds. In this method, the extract was gradient eluted from a C18 column (Waters UPLC BEH C18-2.1x100 mm, 1.7 µm) using water and methanol, containing 0.05% perfluoropentanoic acid (PFPA) and 0.1% formic acid (FA). Another aliquot was also analyzed using acidic positive ion conditions, however it was chromatographically optimized for more hydrophobic compounds. In this method, the extract was gradient eluted from the same afore- mentioned C18 column using methanol, acetonitrile, water, 0.05% PFPA and 0.01% FA and was operated at an overall higher organic content. Another aliquot was analyzed using basic negative ion optimized conditions using a separate dedicated C18 column. The basic extracts were gradient eluted from the column using methanol and water, however with 6.5mM Ammonium Bicarbonate at pH 8. The fourth aliquot was analyzed via negative ionization following elution from a HILIC column (Waters UPLC BEH Amide 2.1x150 mm, 1.7 µm) using a gradient consisting of water and acetonitrile with 10mM Ammonium Formate, pH 10.8. The MS analysis alternated between MS and data-dependent MSn scans using dynamic exclusion. The scan range varied slighted between methods but covered 70-1000 m/z. Raw data files are archived and extracted.

Bioinformatics Methods:

Data Extraction and Compound Identification: Raw data were extracted, peak-identified and QC processed using Metabolon's hardware and software. Compounds were identified by comparison to entries in the library of purified standards or recurrent unknown entities maintained by Metabolon, with the retention time/index (RI), mass to charge ratio (m/z), and chromatographic data (including MS/MS spectral data) on all molecules present in the library. Furthermore, biochemical identifications are based on three criteria: retention index within a narrow RI window of the proposed identification, accurate mass match to the library +/- 10 ppm, and the MS/MS forward and reverse scores between the experimental data and authentic standards. The MS/MS scores are based on a comparison of the ions present in the experimental spectrum to the ions present in the library spectrum.

Metabolite Quantification and Data Normalization: Peaks were quantified using area-under-the-curve (AUC). For studies spanning multiple days, a data normalization step was performed to correct variation resulting from instrument inter-day tuning differences. Essentially, each compound was corrected in run-day blocks by registering the medians to equal one (1.00) and normalizing each data point proportionately. For studies that did not require more than one day of analysis, no normalization is necessary, other than for purposes of data visualization. For urine, data were normalized to urine osmolality to account for differences in urine concentrations (in part a function of fluid intake); serum did not require this normalization. In certain instances, biochemical data may have been normalized to an additional factor (e.g., cell counts, total protein as determined by Bradford assay, osmolality, etc.) to account for differences in metabolite levels due to differences in the amount of material present in each sample.

Statistical Methods:

Metabolon Analysis Methods: Metabolon conducted preliminary analyses that were intended to identify metabolites potentially worth further investigation, but they were not meant to be definitive analyses. Metabolite levels were normalized by dividing each metabolite signal by the median value over all metabolites; the normalized values were then log-transformed. Missing values were imputed with the minimum value observed for each metabolite. Welch's two-sample t-test was used to test for differences in mean metabolite levels between the 2 groups, using a 2-sided test; both p-values and q-values (false discovery rate, FDR) were calculated. T-tests were performed for all patients, and stratified by BMI (<25, 25-29.9, ≥ 30), and stratified by age group (<60, 60-69, ≥ 70). Metabolites identified in the serum or urine samples were sorted by p-value and the 25 lowest p-value metabolites were identified (separately for serum and urine). Principal components analysis was used as an additional unsupervised approach to reduce dimensionality to metabolite clusters and evaluate whether the clusters showed separation between the 2 groups. Random forest analysis was used as a supervised analysis to determine the

metabolites that contributed most strongly to group separation. The "Mean Decrease Accuracy" (MDA) was used to determine which metabolites made the largest contribution to the classification. The MDA is determined by randomly permuting a variable, running the observed values through the trees, and then reassessing the prediction accuracy. If a metabolite is important to the classification, the prediction accuracy will drop after such a permutation, which is recorded as the MDA. Thus, the random forest analysis provided an "importance" rank ordering of metabolites. Metabolon selected the top 30 metabolites in the list as potentially worthy of further investigation.

Johns Hopkins Analysis Methods: Metabolite data as described above were provided in Excel files by Metabolon. To develop a metabolomics signature from the metabolites identified in the samples, dimension reduction was first performed using background filtering to remove features/metabolites with a median signal <0.3. Metabolites that passed the background filter were additionally filtered with a univariate Wilcoxon rank sum test and median fold difference. Features were selected with Wilcoxon p-value < 0.01 and a median fold-difference exceeding the 75th percentile (cases>controls) or less than the 25^{th} percentile (cases<controls). If dimensionality was reduced to 100 or fewer metabolites, those metabolites would be entered into a regularized logistic regression model with an elastic net penalty of $\alpha=0.5$, using the package *glmnet* in R with 10-fold cross validation for model selection. If dimension reduction resulted in more than 100 metabolites, more stringent filtering would be applied by requiring a Wilcoxon p-value adjusted for false discovery < 0.05, although the false discovery rate adjustment can be highly conservative because of the high degree of inter-correlation among metabolites in the same pathways commonly observed in metabolomics data. Once a set if metabolite features was generated from the regularized logistic regression model, the model would be bootstrapped 1000 times, and features that were selected in at least 25% of bootstrapped models would be entered into a logistic regression model using Firth's penalized likelihood, along with clinical features previously associated with upgrading in AS patients (e.g. age, PSA density, number of positive biopsy cores). Variable selection would be based on manual deletion of variables based on p-value and changes to parameter estimates of variables remaining in the model to generate a final signature. The AUC for that model would be compared to the AUC for a model with clinical features alone.

Analysis Results: Serum:

There were 850 known compounds (named biochemicals) identified in the serum samples. There were 169 biochemicals significantly different between cases and controls at a nominal p-value<0.05, with 107 elevated and 62 decreased in cases compared to controls. Table 1 below shows that the relative numbers of metabolites that significantly increased or decreased in cases compared to controls did not differ by BMI group or age group. The fact that within subgroups the number of statistically significant biochemicals in serum is less than the overall number

probably reflects the relative subgroup sample sizes. **Appendix 2** shows all 850 biochemicals ordered by super pathway (e.g. amino acids) and sub-pathway (e.g. glutamate metabolism).

Table 1: Summary of significant associations with upgrading within subgroups of BMI and age,

Statistical Comparisons Welch's Two-Sample t-Test					
Significantly Altered Biochemicals		Total biochemicals $p \leq 0.05$	Biochemicals (↑|↓)	Total biochemicals $0.05 < p < 0.10$	Biochemicals (↑|↓)
	BMI <25	89	50|39	50	29|21
	BMI 25-29.9	92	51|41	36	23|13
	BMI ≥30	48	27|21	50	30|20
GSU(+) GSU(-)	*Age <60*	60	32|28	56	26|30
	Age 60-69	142	78|64	66	33|33
	Age ≥70	49	27|22	53	36|17
	All	169	107|62	47	28|19

Appendix 3 shows the metabolites with the most highly statistically significant (based on nominal p-values) increases or decreases in cases compared to controls. Biological pathways that appeared to show significant alterations between cases and controls (again based on nominal p-values) were as follows:

Gamma-glutamyl amino acids (decreased in cases)
Glutathione metabolism (decreased in cases)
Long chain and polyunsaturated fatty acids (increased in cases)
Ketone bodies (increased in cases)
Sulfated androgenic steroids (increased in cases)
Endocannabinoids (increased in cases)

An initial Random Forest analysis (using median-scaled, log-transformed metabolite levels) by Metabolon demonstrated strong contributions from gamma glutamyl amino acids, lysoplasmalogens, lipids, and glutathione metabolites (see **Appendix 4** which shows the top 30 hits). In agreement with the t-tests, gamma-glutamyl amino acids and long chain and polyunsaturated fatty acids were most highly represented.

The random forest analysis was not intended to develop a metabolomics signature; additional analyses to develop a signature were performed by Dr. Trock. Dimension reduction was first achieved by applying 2 filters: selecting metabolites with mean fold-difference greater than the 75th percentile (fold increases in cases), or less than the 25th percentile (fold decreases in cases), and a Wilcoxon rank sum test with $p<0.01$ to compare each of the 850 metabolites individually between cases and controls. Metabolites passing both filters were selected for multivariable modeling. This reduced dimensionality to 49 metabolites, which were entered into a regularized logistic regression model with an elastic net penalty, using the package *glmnet* in R v3.2.0. The resulting 10-fold cross-validated model retained 20 metabolites, with strong influence of gamma-glutamyl amino acids and long chain and polyunsaturated fatty acids; functional families also identified by the random forest approach. The area under the ROC curve (AUC) was a surprisingly high 0.872. The 20 metabolites and their beta coefficients or ln[odds ratios] were as follows:

Metabolite	Beta coefficient (in standard deviation units)
Gamma-glutamylhistidine	-0.22373397
Gamma-glutamyltryptophan	-0.20602686
3b-hydroxy-5-cholenoic acid	0.11144543
1-(1-enyl-stearoyl)-GPE (P-18:0)	0.11655736
Sphinganine-1-phosphate	0.08488399
1-arachidonylglycerol (20:4)	0.04374527
1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0)	0.31245900
1-arachidonoyl-GPA (20:4)	0.08152260
1,2-dilinoleoyl-GPC (18:2/18:2)	-0.10838159
1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	-0.18229121
Cortisol	-0.07027207
2-hydroxynervonate	0.07248541
Fumarate	0.05213472
1-linoleoyl-GPE (18:2)	-0.05957478
Phosphoethanolamine	0.02015827
1-stearoyl-GPS (18:0)	0.05295649
Acetylcarnitine (C2)	0.05633314
hexanoylglutamine	0.10486277
imidazole-propionate	0.05516056
sphingomyelin (d18:2/18:1)	-0.12219973

Because the cross-validated *glmnet* procedure is fairly robust against over-fitting we examined each of the 20 candidate metabolites for possible confounding that could have spuriously increased the AUC. This revealed a potential sample-age problem. Because of the difference in follow-up inherent in the definition of cases (no Gleason upgrading for 5 or more years) vs.

controls (Gleason upgrading occurring within 3 years), and the difficulty in selecting samples with matched serum and urine close to the date of entry into the AS program, there was a significant difference between cases and controls in the year that the samples were collected, with the result that samples from cases were more recent than those from controls, or equivalently - **sample age** was greater for controls than cases:

Sample collection year	Cases (%)	Controls (%)
2007-2008	20	60
2009-2010	19	25
2011-2012	19	15
2013-2014	32	0
2015-2016	10	0

This prompted testing the correlation of all 850 metabolites against the sample collection year, with the goal of conservatively flagging any metabolite with a correlation p-value $\leq$0.10 for removal from analysis. This resulted in 213 of 850 metabolites identified with a correlation to sample collection year at p$\leq$0.10. For 155 of the 213 metabolites the correlations were positive, i.e. newer samples had higher values. However, 58 of the metabolites were negatively correlated with sample collection year, i.e. older samples had higher values. Fully 18 of the 20 metabolites that were identified in the penalized logistic regression analysis (above) were significantly correlated, positively (12 of 18) or negatively (6 of 18), with sample collection year. Furthermore, the metabolites that were positively correlated with sample collection year (newer samples had higher values) exhibited increases in cases compared to controls, and those that were negatively correlated with sample collection year (older samples had higher values) were decreased in cases compared to controls. Some of the lipids, which were increased in cases compared to controls, exhibited some of the strongest positive correlations with sample age.

After removing the 213 metabolites that were correlated with sample collection year there were 637 metabolites remaining for analysis; these metabolites all had correlation coefficient <0.12 and p-value for the correlation >0.10. Applying the filters described above, we restricted analysis to metabolites with median scaled expression >0.25, and with median-fold difference greater than the 75th percentile (fold increases) or less than the 25th percentile (fold decreases), with nominal Wilcoxon p-value <0.01. This resulted in 18 metabolites passing the filters, which were entered into the *glmnet* with 10-fold cross-validation, resulting in the following 14 metabolites with statistically significant independent associations with Gleason score upgrading:

Metabolite	Beta coefficient (in standard deviation units)
1-linoleoyl-GPE (18:2)	-0.247943921
3b-hydroxy-5-cholenoic acid	0.254892475
3beta-hydroxy-5-cholestenoate	0.056813824
acetoacetate	0.071682341
adenosine	0.228857866
cysteine-glutathione disulfide	-0.230351193
gamma-glutamyl-epsilon-lysine	-0.146676774
gamma-glutamylglycine	-0.222628371
gamma-glutamylvaline	-0.132749603
hexanoylglycine	0.202107329
linoleoyl-linoleoyl-glycerol (18:2/18:2) [1]	-0.062485752
N-oleoyltaurine	0.155392095
palmitoleoyl-linoleoyl-glycerol (16:1/18:2) [1]	-0.193173689
sucrose	-0.027676272

Despite restricting analysis to metabolites that were not correlated with sample age, the model still shows strong representation of lipids (8 metabolites) and gamma-glutamyl amino acids (3 metabolites); there was 1 each of nucleotides, amino acids, and carbohydrates. This signature had AUC = 0.793. The strong confounding associated with metabolites correlated with sample age can be seen in that only 4 metabolites – 3b-hydroxy-5-cholenoic acid, cysteine-glutathione disulfide, gamma-glutamylglycine, and sucrose – were common to both the initial random forest analysis (before exclusion of correlated metabolites) and the regularized logistic regression with elastic net penalty (excluding correlated metabolites).

Analysis Results: Urine

There were 691 known compounds (named biochemical) identified in the serum samples. There were 103 biochemicals significantly different between cases and controls at a nominal p-value<0.05, with 28 elevated and 75 decreased in cases compared to controls. Table 2 below shows the relative numbers of metabolites that significantly increased or decreased in cases compared to controls. Unlike the associations in serum, these show potential differences in the numbers of increases relative to decreases across BMI age categories, suggesting potential interactions. **Appendix 5** shows all 691 biochemicals ordered by super pathway (e.g. amino acids) and sub-pathway (e.g. glutamate metabolism).

Appendix 6 compares metabolites in urine and serum that were statistically significantly (based on nominal p-values) increased or decreased in cases vs. controls. From the figure it appears that only the gamma-glutamyl amino acids are strongly represented in both sample matrices, but note

that lipids are not excreted in urine. Biological pathways that appeared to show significant alterations between cases and controls (again based on nominal p-values) were as follows:

Mitochondrial tricarboxylic acid (Krebs) cycle (increases and decreases in cases)
Gamma-glutamyl amino acids (decreased in cases)
Acylglutamines (increased in cases)

Table 2: Summary of significant associations with upgrading within subgroups of BMI and age,

Statistical Comparisons Welch's Two-Sample t-Test, Osmolality Normalized Data					
Significantly Altered Biochemicals		Total biochemicals $p \leq 0.05$	Biochemicals (↑↓)	Total biochemicals $0.05 < p < 0.10$	Biochemicals (↑↓)
	BMI <25	41	28|13	28	20|8
	BMI 25-29.9	60	4|56	46	13|33
	BMI ≥30	23	4|19	23	5|18
GSU(+) GSU(-)	*Age <60*	47	1|46	32	0|32
	Age 60-69	91	10|81	59	6|53
	Age ≥70	42	26|16	46	41|5
	All	103	28|75	52	23|29

An initial Random Forest analysis of urine samples (using median-scaled, log-transformed metabolite levels) by Metabolon demonstrated very little overlap with the profile observed in serum, with gamma glutamyl amino acids as the main pathway common to both sample types, as noted above (see **Appendix 7** which shows the top 30 hits). As with serum, a substantial number – 146 - of urine metabolites were correlated with sample age at $p \leq 0.10$. The remaining 545 metabolites that were not correlated with sample age (in addition to p-value >0.10 all had a correlation coefficient with absolute value <0.12) were entered into the analysis pipeline.

Only 1 metabolite (4-hydroxyphenylpyruvate) met the filter restricted to metabolites with median scaled expression >0.25, and with median-fold difference greater than the 75th percentile (fold increases) or less than the 25th percentile (fold decreases), with nominal Wilcoxon p-value <0.01. When the Wilcoxon criterion of the filter was reduced to nominal p-value <0.05 there were 10 metabolites that passed the filters, which were entered into *glmnet* with 10-fold cross-

validation, resulting in the following 9 metabolites with statistically significant independent associations with Gleason score upgrading):

Metabolite	Beta coefficient (in standard deviation units)
(Intercept)	-0.35713856
3-hydroxyphenylacetatoylcarnitine	0.14644970
4-hydroxyhippurate	-0.25816265
4-hydroxyphenylpyruvate	0.22503759
guanosine	0.29288687
N3-methyluridine	0.55895061
salicylate	0.21456012
sinapate	-0.22603590
umbelliferone sulfate	-0.05509034
vanillic alcohol sulfate	-0.28401176>

Four metabolites were xenobiotics, 3 were amino acids, and 2 were nucleotides. The signature had AUC = 0.711. The strong confounding associated with metabolites correlated with sample age can be seen in that only 2 metabolites – 4-hydroxyphenylpyruvate and N3-methylurine – were common to both the initial random forest analysis (before exclusion of correlated metabolites) and the regularized logistic regression with elastic net penalty (excluding correlated metabolites). No metabolites were common to both the serum and urine penalized regression signatures after excluding correlated metabolites.

Next Steps

Analysis of both the serum and urine data continues. In order to develop robust serum and urine signatures and determine whether any of the metabolites provide additional predictive value beyond that associated with clinical variables, we will bootstrap the serum and urine regularized logistic regression models 1000 times. Then, from each set of bootstrapped models we will select metabolites that are present in at least 25% of bootstrapped models. A "baseline" clinical model will be generated from clinical and pathology attributes that have previously been shown to be associated with upgrading in active surveillance (3). Then, the metabolites selected from the bootstrapped samples will be added to the model, and non-significant terms will be backward eliminated using a manual approach based on p-value $\geq$0.05 and change in parameters remaining in the model. Modeling will be performed with logistic regression using Firth's penalized maximum likelihood approach to reduce sparse data bias (4). The final model of metabolites plus clinical variables will be compared to the baseline model based on the c-index, calibration curves, and decision curve analysis (5). Based on the results in Table 2, we will also evaluate potential interactions with age or BMI in the urine model.

In addition to developing a prognostic signature, we will also perform pathway analyses to identify potential mechanisms underlying associations between metabolites and upgrading. In particular the strong representation of lipids and gamma-glutamyl amino acids in the serum analysis may indicate importance of particular pathways and provide hypotheses for testing in vitro or in tumor samples.

Finally, we have extensive epidemiologic and dietary (food frequency) data from the patients in this study. We will determine if any of the metabolites in the final models are associated with particular nutrients, food groups, or lifestyle factors (e.g. smoking, physical activity).

We also plan to explore in more detail the metabolites that were correlated with sample age, to investigate whether this is an important source of bias to consider in metabolomic analyses.

We anticipate that the above analyses will be completed by June 2019. Each of the above analyses are likely to generate at least one manuscript for a peer-reviewed journal.

REPORTABLE OUTCOMES

None, but we are getting close.

ACHIEVEMENT OF TASKS/SUBTASKS DEFINED IN STATEMENT OF WORK (SOW)

The SOW was revised on July 21, 2014 to reflect changes in the study aims due to delays and changes in scientific knowledge of prostate cancer metabolomics during that time (see above summaries of progress during Years 3 and 4). The revised SOW and completion of tasks and subtasks is below.

Specific Aim 1: Compare established metabolic signature of aggressive prostate cancer in serum and urine from 50 patients with Gleason 6 vs. 50 patients with Gleason 7 tumors	**Task/Subtask Completion**
Major Task 1: Sample accrual and analysis	
Subtask 1: Identify eligible patients with matched serum and urine samples, who underwent prostatectomy with pure Gleason 6 or pure Gleason 7 tumor.	Samples identified. Because the original Aim 1 to first identify a profile in tumor tissue was no longer relevant, we increased the sample size from 50 patients per group to 100 per group.
Subtask 2: Aliquot serum and urine samples	Samples were aliquoted for totaling 80 patients with matched serum and urine, 21 patients with serum, and 6 patients with urine only (total 187 patients).
Subtask 3: Send samples to Metabolon, Inc. for analysis	Samples were sent to Metabolon
Milestone(s) Achieved: 100 patients with samples identified	Samples from 187 of target 200 patients were sent to Metabolon
Major Task 2: Data analysis	
Subtask 1: Receive metabolomic profile data from subcontractor Metabolon, Inc.	Discussion with Metabolon scientists focused on issues with the above 187 samples (discussed in Summary of Year 4 above) led to decision not to analyze those samples, but to proceed directly to the 100 active surveillance patients without upgrade and 100 with upgrade targeted for Aim 2, and perform discovery in those samples. A pilot study was conducted to determine the effect on metabolite detection of PCA3 buffer in the urine sample tubes. The study showed some decrease in detection, but relatively minor.
Subtask 2: Biostatistical and bioinformatics analysis of data	
Milestone(s) Achieved:	Study refined to avoid potential for bias. Pilot study confirms acceptability of urine samples stored with PCA3 buffer.
Specific Aim 2: Using metabolomics profile as in Aim 1, compare serum and urine from 100 men in Active Surveillance with biopsy progression vs. 100 men without biopsy progression for ≥5 years	
Major Task 3: Sample accrual and analysis	
Subtask 1: Identify 200 patients meeting Aim 2 criteria with available serum and urine	Samples identified.
Subtask 2: Aliquot samples	Samples aliquoted.

Subtask 3: Send samples to Metabolon, Inc.	Samples sent to Metabolon.
Milestone(s) Achieved: 250 potentially eligible patients identified, 200 selected.	Requisite samples received at Metabolon, ready for metabolomics analysis.
Major Task 4: Data analysis	
Subtask 1: Receive metabolomic profile data from subcontractor Metabolon, Inc.	Data received. Similar analysis methods applied to both serum and urine metabolomnic data.
Subtask 2: Biostatistical and bioinformatics analysis of data	Initial dimension reduction performed. Regularized logistic regression modeling performed. Tentative metabolite signatures identified. Final signature development using bootstrapping and combining metabolites with clinical variables remains to be completed.
Milestone(s) Achieved:	Metabolomic analyses completed. Data from Metabolon Inc. received at Johns Hopkins. Initial development of metabolomics signatures for urine and serum completed.
Major Task 5: Prepare & submit manuscript(s)	
	Final biostatistical analyses and model refinement remain to be completed. Pathway analyses, and association of metabolite signatures with dietary and lifestyle variables also planned. Initial manuscripts of metabolomics signatures in serum and urine projected for submission by June 2019.

CONCLUSIONS

Metabolomic analysis of serum and urine from men with low and very low risk prostate cancer managed by active surveillance is a feasible approach to discover biomarkers associated with risk of upgrading. If a signature can be validated it could be used to augment current eligibility criteria for enrollment in active surveillance, and could be used during follow-up to identify men at risk of progression who may not be detected at surveillance biopsy due to sampling errors.

The study reported herein encountered a number of logistical difficulties detailed in previous Progress Reports and in the current report. These resulted in delay to the study and a study population susceptible to confounding associated with sample age. Despite this, the study was fully enrolled and metabolomics analyses and initial biostatistical analyses completed. The

results thus far suggest that informative metabolites have been identified. Further analyses to be performed by Dr. Trock will complete the study aims.

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APPENDICES

1. List of abbreviations and acronyms.

2. PDF file of 850 biochemicals detected in serum. *This file submitted as a separate file due to large size.*

3. Most highly statistically significant metabolites in serum.

4. Random forest plot of serum metabolites.

5. PDF file of 691 biochemicals detected in urine. *This file submitted as a separate file due to large size.*

6. Comparison of most significant metabolites in urine vs. serum.

7. Random forest plot of urine metabolites

8. Abstracts, publications and manuscripts in preparation

9. Personnel receiving pay from this negotiated effort

Appendix 1: List of abbreviations and acronyms

AS	active surveillance
AUC	area under the curve
JHU	Johns Hopkins University
MTA	Material Transfer Agreement
PCBN	Prostate Cancer Biorepository Network
RAL	robot assisted laparoscopic prostatectomy
ROC	receiver operating characteristic
RRP	radical retropubic open prostatectomy
TURP	transurethral resection of the prostate

Appendix 2: See attached PDF file “Appendix 2 - serum metabolites.pdf”

Johns Hopkins University JHOP-0801-16VW

Gleason score stratification in prostate cancer: serum

Biochemicals profiled in this study, by Super Pathway & Sub-Pathway

Pathway Sort Order	Super Pathway	Sub Pathway	Biochemical Name	KEGG	HMDB	PubChem
1		Glycine, Serine and Threonine Metabolism	glycine	C00037	HMDB00123	750
2			N-acetylglycine		HMDB00532	10972
4			sarcosine	C00213	HMDB00271	1088
5			dimethylglycine	C01026	HMDB00092	673
6			betaine	C00719	HMDB00043	247
9			serine	C00065	HMDB00187	5951
10			N-acetylserine		HMDB02931	65249
16			threonine	C00188	HMDB00167	6288
17			N-acetylthreonine			152204
28		Alanine and Aspartate Metabolism	alanine	C00041	HMDB00161	5950
30			N-acetylalanine	C02847	HMDB00766	88064
33			N-carbamoylalanine			426409
34			aspartate	C00049	HMDB00191	5960
35			N-acetylaspartate (NAA)	C01042	HMDB00812	65065
38			asparagine	C00152	HMDB00168	6267
40		Glutamate Metabolism	glutamate	C00025	HMDB00148	611
41			glutamine	C00064	HMDB00641	5961
42			N-acetylglutamate	C00624	HMDB01138	70914
43			N-acetylglutamine	C02716	HMDB06029	182230
45			4-hydroxyglutamate	C03079	HMDB01344	439902
47			gamma-carboxyglutamate		HMDB41900	40772
48			glutamate, gamma-methyl ester		HMDB61715	68662
49			pyroglutamine*			134508
50			N-acetyl-aspartyl-glutamate (NAAG)	C12270	HMDB01067	5255
51			beta-citrylglutamate	C20775		72715786
53			carboxyethyl-GABA		HMDB02201	2572
55			2-pyrrolidinone		HMDB02039	12025
57			S-1-pyrroline-5-carboxylate	C04322	HMDB01301	1196
62		Histidine Metabolism	histidine	C00135	HMDB00177	6274
63			1-methylhistidine	C01152	HMDB00001	92105
64			3-methylhistidine	C01152	HMDB00479	64969
65			N-acetylhistidine	C02997	HMDB32055	75619
66			N-acetyl-3-methylhistidine*			193270
67			N-acetyl-1-methylhistidine*			193270
68			hydantoin-5-propionic acid	C05565	HMDB01212	782
69			trans-urocanate	C00785	HMDB00301	736715
71			imidazole propionate		HMDB02271	70630
72			formiminoglutamate	C00439	HMDB00854	439233
73			imidazole lactate	C05568	HMDB02320	440129
76			N-acetylcarnosine		HMDB12881	9903482
80			1-methylimidazoleacetate	C05828	HMDB02820	75810
81			4-imidazoleacetate	C02835	HMDB02024	96215
87		Lysine Metabolism	lysine	C00047	HMDB00182	5962
88			N2-acetyllysine	C12989	HMDB00446	92907
89			N6-acetyllysine	C02727	HMDB00206	92832
94			N6,N6,N6-trimethyllysine	C03793	HMDB01325	440120
95			5-hydroxylysine	C16741	HMDB00450	1029
96			5-(galactosylhydroxy)-L-lysine			
98			2-aminoadipate	C00956	HMDB00510	469

102			glutarylcarnitine (C5-DC)		HMDB13130	71464488
106			pipecolate	C00408	HMDB00070	849
107			6-oxopiperidine-2-carboxylate		HMDB61705	3014237
109			N-acetyl-cadaverine		HMDB02284	189087
111			N-trimethyl 5-aminovalerate			
112		Phenylalanine Metabolism	phenylalanine	C00079	HMDB00159	6140
113			N-acetylphenylalanine	C03519	HMDB00512	74839
116			phenylpyruvate	C00166	HMDB00205	997
117			phenyllactate (PLA)	C05607	HMDB00779	3848
121			phenylacetate	C07086	HMDB00209	999
122			4-hydroxyphenylacetate	C00642	HMDB00020	127
128		Tyrosine Metabolism	tyrosine	C00082	HMDB00158	6057
129			N-acetyltyrosine		HMDB00866	68310
136			4-hydroxyphenylpyruvate	C01179	HMDB00707	979
138			4-hydroxyphenylacetatoylcarnitine			
139			3-(4-hydroxyphenyl)lactate	C03672	HMDB00755	9378
143			phenol sulfate	C02180	HMDB60015	74426
153			vanillactate		HMDB00913	160637
154			vanillylmandelate (VMA)	C05584	HMDB00291	1245
156			3-methoxytyrosine		HMDB01434	1670
158			3-methoxytyramine sulfate			
162			homovanillate (HVA)	C05582	HMDB00118	1738
171			gentisate	C00628	HMDB00152	3469
172			5-hydroxymethyl-2-furoic acid	C20448	HMDB02432	80642
173			2-hydroxyphenylacetate	C05852	HMDB00669	11970
176			dopamine 4-sulfate	C13691	HMDB04148	123932
177			dopamine 3-O-sulfate	C13690	HMDB06275	122136
178			p-cresol-glucuronide*		HMDB11686	154035
179			tyramine O-sulfate		HMDB06409	153005
180			N-formylphenylalanine			759256
181			vanillic alcohol sulfate			
184			3,4-dihydroxyphenylacetate sulfate			193283
191			catechol glucuronide			75124209
196			thyroxine	C01829	HMDB01918	5819
200		Tryptophan Metabolism	tryptophan	C00078	HMDB00929	6305
201			N-acetyltryptophan	C03137	HMDB13713	700653
207			C-glycosyltryptophan			10981970
209			tryptophan betaine	C09213	HMDB61115	442106
211			kynurenine	C00328	HMDB00684	161166
213			N-acetylkynurenine (2)			
215			kynurenate	C01717	HMDB00715	3845
217			N-formylanthranilic acid	C05653	HMDB04089	101399
218			anthranilate	C00108	HMDB01123	227
221	Amino Acid		xanthurenate	C02470	HMDB00881	5699
223			picolinate	C10164	HMDB02243	1018
224			serotonin	C00780	HMDB00259	5202
227			5-hydroxyindoleacetate	C05635	HMDB00763	1826
231			indolelactate	C02043	HMDB00671	92904
232			indoleacetate	C00954	HMDB00197	802
234			indolepropionate		HMDB02302	3744
236			indoleacetylglutamine		HMDB13240	25200879
239			indole-3-carboxylic acid	C19837	HMDB03320	69867
244			3-indoxyl sulfate		HMDB00682	10258
246			5-bromotryptophan			96735
247			leucine	C00123	HMDB00687	6106
248			N-acetylleucine	C02710	HMDB11756	70912
250			4-methyl-2-oxopentanoate	C00233	HMDB00695	70

253			alpha-hydroxyisocaproate	C03264	HMDB00746	83697
256			isovalerate (i5:0)	C08262	HMDB00718	10430
257			isovalerylglycine		HMDB00678	546304
258			isovalerylcarnitine (C5)		HMDB00688	6426851
265			beta-hydroxyisovalerate		HMDB00754	69362
268			3-methylglutaconate		HMDB00522	1551553
272			3-methylglutarylcarnitine (2)		HMDB00552	128145
281			isoleucine	C00407	HMDB00172	6306
284			3-methyl-2-oxovalerate	C00671	HMDB03736	47
285			alpha-hydroxyisovalerate		HMDB00407	99823
287		Leucine, Isoleucine and Valine Metabolism	2-methylbutyrylcarnitine (C5)		HMDB00378	6426901
289			tiglylcarnitine (C5:1-DC)		HMDB02366	22833596
292			3-hydroxy-2-ethylpropionate		HMDB00396	188979
294			ethylmalonate		HMDB00622	11756
295			methylsuccinate		HMDB01844	10349
296			methylsuccinoylcarnitine (1)			
301			valine	C00183	HMDB00883	6287
302			N-acetylvaline		HMDB11757	66789
304			3-methyl-2-oxobutyrate	C00141	HMDB00019	49
305			2-hydroxy-3-methylvalerate		HMDB00317	164623
307			isobutyrylcarnitine (C4)		HMDB00736	168379
308			isobutyrylglycine		HMDB00730	10855600
309			3-hydroxyisobutyrate	C06001	HMDB00336	87
310			2,3-dihydroxy-2-methylbutyrate		HMDB29576	301941
313			methionine	C00073	HMDB00696	6137
314			N-acetylmethionine	C02712	HMDB11745	448580
315			N-formylmethionine	C03145	HMDB01015	439750
316			S-methylmethionine	C05319	HMDB38670	458
317			methionine sulfone			69961
318			methionine sulfoxide	C02989	HMDB02005	158980
319			N-acetylmethionine sulfoxide			193368
324			S-adenosylhomocysteine (SAH)	C00021	HMDB00939	439155
328			cystathionine	C02291	HMDB00099	439258
329			alpha-ketobutyrate	C00109	HMDB00005	58
330		Methionine, Cysteine, SAM and Taurine Metabolism	cysteine	C00097	HMDB00574	5862
332			S-methylcysteine		HMDB02108	24417
333			S-methylcysteine sulfoxide		HMDB29432	82142
335			cysteine s-sulfate	C05824	HMDB00731	115015
336			cystine	C00491	HMDB00192	67678
340			cysteine sulfinic acid	C00606	HMDB00996	109
341			hypotaurine	C00519	HMDB00965	107812
342			taurine	C00245	HMDB00251	1123
343			N-acetyltaurine			159864
344			N-methyltaurine			7882
347			3-sulfo-L-alanine	C00506	HMDB02757	72886
349			arginine	C00062	HMDB00517	232
351			urea	C00086	HMDB00294	1176
353			ornithine	C00077	HMDB03374	6262
356			2-oxoarginine*	C03771	HMDB04225	558
357			citrulline	C00327	HMDB00904	9750
358			homoarginine	C01924	HMDB00670	9085
359			homocitrulline	C02427	HMDB00679	65072
360			proline	C00148	HMDB00162	145742
363		Urea cycle; Arginine and Proline Metabolism	dimethylarginine (SDMA + ADMA)	C03626	HMDB01539	123831
364			N-acetylarginine	C02562	HMDB04620	67427
365			N-acetylcitrulline	C15532	HMDB00856	656979
366			N-acetylproline			322640
367			N-delta-acetylornithine			9920500

369			N2,N5-diacetylornithine			10398396
371			trans-4-hydroxyproline	C01157	HMDB00725	5810
373			pro-hydroxy-pro		HMDB06695	11673055
376			N-methylproline			557
379			argininate*		HMDB03148	160437
382		Creatine Metabolism	guanidinoacetate	C00581	HMDB00128	763
383			creatine	C00300	HMDB00064	586
384			creatinine	C00791	HMDB00562	588
395		Polyamine Metabolism	acisoga			129397
399			5-methylthioadenosine (MTA)	C00170	HMDB01173	439176
400			N-acetylputrescine	C02714	HMDB02064	122356
404			4-acetamidobutanoate	C02946	HMDB03681	18189
406			(N(1) + N(8))-acetylspermidine			
407		Guanidino and Acetamido Metabolism	1-methylguanidine	C02294	HMDB01522	10111
408			4-guanidinobutanoate	C01035	HMDB03464	500
409			guanidinosuccinate	C03139	HMDB03157	97856
414		Glutathione Metabolism	cysteine-glutathione disulfide		HMDB00656	4247235
417			cysteinylglycine	C01419	HMDB00078	439498
418			cys-gly, oxidized			333293
419			5-oxoproline	C01879	HMDB00267	7405
420			2-aminobutyrate	C02261	HMDB00650	439691
422			2-hydroxybutyrate/2-hydroxyisobutyrate			
431	Peptide	Gamma-glutamyl Amino Acid	gamma-glutamylalanine		HMDB29142	440103
433			gamma-glutamylglutamate	C05282	HMDB11737	92865
434			gamma-glutamylglutamine	C05283	HMDB11738	150914
435			gamma-glutamylglycine		HMDB11667	165527
436			gamma-glutamylhistidine			7017195
437			gamma-glutamylisoleucine*		HMDB11170	14253342
438			gamma-glutamylleucine		HMDB11171	151023
439			gamma-glutamyl-alpha-lysine			65254
440			gamma-glutamyl-epsilon-lysine		HMDB03869	7015685
441			gamma-glutamylmethionine		HMDB29155	7009567
442			gamma-glutamylphenylalanine		HMDB00594	111299
443			gamma-glutamylthreonine		HMDB29159	76078708
444			gamma-glutamyltryptophan		HMDB29160	3989307
445			gamma-glutamyltyrosine		HMDB11741	94340
446			gamma-glutamylvaline		HMDB11172	7015683
447			gamma-glutamyl-2-aminobutyrate			
597		Dipeptide	glycylvaline		HMDB28854	97417
602			histidylalanine		HMDB28878	351667
621			isoleucylglycine		HMDB28907	342532
634			leucylalanine		HMDB28922	259321
639			leucylglycine		HMDB28929	79070
708			prolylglycine		HMDB11178	6426709
749			threonylphenylalanine		HMDB29068	4099799
792			valylglycine		HMDB29127	136487
817		Polypeptide	bradykinin, des-arg(9)	C00306	HMDB04246	105044
821			HWESASXX*			
849		Fibrinogen Cleavage Peptide	ADSGEGDFXAEGGGVR*			16133137
850			DSGEGDFXAEGGGVR*			
851			ADpSGEGDFXAEGGGVR*			
862		Acetylated Peptides	phenylacetylcarnitine			101724840
864			phenylacetylglutamate		HMDB59772	11579826
865			phenylacetylglutamine	C04148	HMDB06344	92258
866			4-hydroxyphenylacetylglutamine			
867			phenylacetylglycine	C05598	HMDB00821	68144
875			1,5-anhydroglucitol (1,5-AG)	C07326	HMDB02712	64960

878		Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	glucose	C00031	HMDB00122	79025
894			pyruvate	C00022	HMDB00243	1060
895			lactate	C00186	HMDB00190	612
898			glycerate	C00258	HMDB00139	752
913		Pentose Metabolism	ribitol	C00474	HMDB00508	6912
914			ribonate	C01685	HMDB00867	5460677
918			xylose	C00181	HMDB00098	135191
920			arabinose	C00216	HMDB00646	66308
935			arabitol/xylitol	C01904		6912
937			arabonate/xylonate			
947	Carbohydrate	Glycogen Metabolism	maltose	C00208	HMDB00163	10991489
969		Disaccharides and Oligosaccharides	sucrose	C00089	HMDB00258	5988
986		Fructose, Mannose and Galactose Metabolism	fructose	C00095	HMDB00660	5984
991			mannitol/sorbitol	C00794	HMDB00247	5780
992			mannose	C00159	HMDB00169	18950
1011			galactonate	C00880	HMDB00565	128869
1038		Aminosugar Metabolism	glucuronate	C00191	HMDB00127	444791
1049			N-acetylneuraminate	C00270	HMDB00230	439197
1060			N-acetylglucosaminylasparagine	C04540	HMDB00489	123826
1061			erythronate*		HMDB00613	2781043
1063			N-acetylglucosamine/N-acetylgalactosamine		HMDB00215	24139
1064		Advanced Glycation End-product	N6-carboxymethyllysine			123800
1068	Energy	TCA Cycle	citrate	C00158	HMDB00094	311
1070			aconitate [cis or trans]			
1074			alpha-ketoglutarate	C00026	HMDB00208	51
1076			succinylcarnitine (C4-DC)		HMDB61717	71464481
1077			succinate	C00042	HMDB00254	1110
1078			fumarate	C00122	HMDB00134	444972
1079			malate	C00149	HMDB00156	525
1088			citraconate/glutaconate			
1089			2-methylcitrate/homocitrate			
1093		Oxidative Phosphorylation	phosphate	C00009	HMDB01429	1061
1095		Fatty Acid Synthesis	malonylcarnitine		HMDB02095	22833583
1096			malonate	C00383	HMDB00691	867
1110		Short Chain Fatty Acid	valerate	C00803	HMDB00892	7991
1111		Medium Chain Fatty Acid	caproate (6:0)	C01585	HMDB00535	8892
1112			heptanoate (7:0)	C17714	HMDB00666	8094
1113			caprylate (8:0)	C06423	HMDB00482	379
1115			caprate (10:0)	C01571	HMDB00511	2969
1117			10-undecenoate (11:1n1)	C13910	HMDB33724	14891
1118			laurate (12:0)	C02679	HMDB00638	3893
1119			5-dodecenoate (12:1n7)		HMDB00529	5312378
1121		Long Chain Fatty Acid	myristate (14:0)	C06424	HMDB00806	11005
1122			myristoleate (14:1n5)	C08322	HMDB02000	5281119
1125			pentadecanoate (15:0)	C16537	HMDB00826	13849
1126			palmitate (16:0)	C00249	HMDB00220	985
1127			palmitoleate (16:1n7)	C08362	HMDB03229	445638
1129			margarate (17:0)		HMDB02259	10465
1130			10-heptadecenoate (17:1n7)		HMDB60038	5312435
1132			stearate (18:0)	C01530	HMDB00827	5281
1134			oleate/vaccenate (18:1)			
1141			nonadecanoate (19:0)	C16535	HMDB00772	12591
1142			10-nonadecenoate (19:1n9)		HMDB13622	5312513
1145			arachidate (20:0)	C06425	HMDB02212	10467
1148			eicosenoate (20:1)	C16526	HMDB02231	5282768
1153			erucate (22:1n9)	C08316	HMDB02068	5281116
1160			hexadecadienoate (16:2n6)		HMDB00477	
1164			stearidonate (18:4n3)	C16300	HMDB06547	5312508

1165			eicosapentaenoate (EPA; 20:5n3)	C06428	HMDB01999	446284
1166			docosapentaenoate (n3 DPA; 22:5n3)	C16513	HMDB06528	6441454
1167			docosahexaenoate (DHA; 22:6n3)	C06429	HMDB02183	445580
1168			docosatrienoate (22:3n3)	C16534	HMDB02823	5312556
1171			nisinate (24:6n3)		HMDB02007	11792612
1172		Polyunsaturated Fatty Acid (n3 and n6)	linoleate (18:2n6)	C01595	HMDB00673	5280450
1174			linolenate [alpha or gamma; (18:3n3 or 6)]	C06426	HMDB03073	5280934
1176			dihomo-linolenate (20:3n3 or n6)	C03242	HMDB02925	5280581
1177			arachidonate (20:4n6)	C00219	HMDB01043	444899
1178			adrenate (22:4n6)	C16527	HMDB02226	5497181
1179			docosapentaenoate (n6 DPA; 22:5n6)	C16513	HMDB01976	6441454
1180			docosadienoate (22:2n6)	C16533	HMDB61714	5282807
1181			dihomo-linoleate (20:2n6)	C16525	HMDB05060	6439848
1238		Fatty Acid, Branched	15-methylpalmitate			17903417
1241			17-methylstearate (i19:0)		HMDB37397	3083779
1251		Fatty Acid, Dicarboxylate	glutarate (pentanedioate)	C00489	HMDB00661	743
1255			2-hydroxyglutarate	C02630	HMDB00606	43
1256			4-hydroxy-2-oxoglutaric acid	C01127	HMDB02070	599
1259			2-hydroxyadipate	C02360	HMDB00321	193530
1260			3-methyladipate		HMDB00555	12292
1262			maleate	C01384	HMDB00176	444266
1263			pimelate (heptanedioate)	C02656	HMDB00857	385
1265			suberate (octanedioate)	C08278	HMDB00893	10457
1268			azelate (nonanedioate)	C08261	HMDB00784	2266
1269			sebacate (decanedioate)	C08277	HMDB00792	5192
1272			dodecanedioate	C02678	HMDB00623	12736
1273			tetradecanedioate		HMDB00872	13185
1274			hexadecanedioate	C19615	HMDB00672	10459
1275			octadecanedioate		HMDB00782	70095
1276			eicosanodioate			75502
1277			docosadioate	C19625		244872
1278			3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)		HMDB61112	123979
1309		Fatty Acid, Amino	2-aminoheptanoate			227939
1314			2-aminooctanoate		HMDB00991	69522
1325		Fatty Acid Metabolism (also BCAA Metabolism)	butyrylcarnitine (C4)	C02862	HMDB02013	439829
1328			propionylcarnitine (C3)	C03017	HMDB00824	107738
1329			propionylglycine		HMDB00783	98681
1332		Fatty Acid Metabolism (Acyl Glutamine)	hexanoylglutamine			
1337		Fatty Acid Metabolism(Acyl Glycine)	hexanoylglycine		HMDB00701	99463
1341			N-palmitoylglycine		HMDB13034	151008
1346		Fatty Acid Metabolism(Acyl Carnitine)	acetylcarnitine (C2)	C02571	HMDB00201	1
1347			3-hydroxybutyrylcarnitine (1)		HMDB13127	53481617
1348			3-hydroxybutyrylcarnitine (2)		HMDB13127	
1350			hexanoylcarnitine (C6)		HMDB00705	6426853
1351			octanoylcarnitine (C8)	C02838	HMDB00791	123701
1353			decanoylcarnitine (C10)		HMDB00651	10245190
1355			cis-4-decenoylcarnitine (C10:1)			
1356			laurylcarnitine (C12)		HMDB02250	10427569
1357			myristoylcarnitine (C14)		HMDB05066	6426854
1359			palmitoylcarnitine (C16)	C02990	HMDB00222	461
1360			palmitoleoylcarnitine (C16:1)*			71464547
1361			stearoylcarnitine (C18)		HMDB00848	6426855
1362			linoleoylcarnitine (C18:2)*		HMDB06469	6450015
1363			linolenoylcarnitine (C18:3)*			
1364			oleoylcarnitine (C18:1)		HMDB05065	6441392
1366			myristoleoylcarnitine (C14:1)*			90659872
1367			suberoylcarnitine (C8-DC)			

1369			adipoylcarnitine (C6-DC)		HMDB61677	71296139
1372			pimeloylcarnitine/3-methyladipoylcarnitine (C7-DC)			
1374			arachidoylcarnitine (C20)*		HMDB06460	
1375			arachidonoylcarnitine (C20:4)			
1378			dihomo-linolenoylcarnitine (20:3n3 or 6)*			
1379			dihomo-linoleoylcarnitine (C20:2)*			
1380			eicosenoylcarnitine (C20:1)*			
1387			lignoceroylcarnitine (C24)*			
1388			margaroylcarnitine*		HMDB06210	
1389			nervonoylcarnitine (C24:1)*			
1390			cerotoylcarnitine (C26)*		HMDB06347	
1391			ximenoylcarnitine (C26:1)*			
1395		Carnitine Metabolism	deoxycarnitine	C01181	HMDB01161	134
1396			carnitine	C00318	HMDB00062	10917
1399		Ketone Bodies	acetoacetate	C00164	HMDB00060	96
1401			3-hydroxybutyrate (BHBA)	C01089	HMDB00357	441
1404		Fatty Acid Metabolism (Acyl Choline)	palmitoylcholine			151731
1405			oleoylcholine			
1407			dihomo-linolenoyl-choline			
1408			linoleoylcholine*			
1409			stearoylcholine*			
1410			docosahexaenoylcholine			
1411			arachidonoylcholine			
1416		Fatty Acid, Monohydroxy	2-hydroxyoctanoate		HMDB02264	94180
1417			2-hydroxydecanoate			21488
1420			2-hydroxynervonate*			5312783
1422			2-hydroxypalmitate		HMDB31057	92836
1424			2-hydroxystearate	C03045		69417
1430			3-hydroxyhexanoate			151492
1431			3-hydroxyoctanoate		HMDB01954	26613
1432			3-hydroxydecanoate		HMDB02203	26612
1433			3-hydroxysebacate		HMDB00350	3017884
1434			3-hydroxylaurate		HMDB00387	94216
1439			5-hydroxyhexanoate		HMDB00525	170748
1450			13-HODE + 9-HODE			43013
1465			9-hydroxystearate		HMDB61661	9570127
1468			2-hydroxylaurate			97783
1473		Fatty Acid, Dihydroxy	12,13-DiHOME	C14829	HMDB04705	10236635
1474			9,10-DiHOME	C14828	HMDB04704	9966640
1565		Endocannabinoid	oleoyl ethanolamide		HMDB02088	5283454
1576			N-oleoyltaurine			6437033
1577			N-stearoyltaurine			168274
1580			linoleoyl ethanolamide		HMDB12252	5283446
1591			N-palmitoylserine			6453686
1592			N-oleoylserine			
1594		Inositol Metabolism	myo-inositol	C00137	HMDB00211	892
1595			chiro-inositol	C19891	HMDB34220	
1623		Phospholipid Metabolism	choline	C00114	HMDB00097	305
1624			choline phosphate	C00588	HMDB01565	1014
1627			glycerophosphorylcholine (GPC)	C00670	HMDB00086	71920
1629			phosphoethanolamine	C00346	HMDB00224	1015
1631			glycerophosphoethanolamine	C01233	HMDB00114	123874
1633			glycerophosphoinositol*			167572
1634			trimethylamine N-oxide	C01104	HMDB00925	1145
1650			1-myristoyl-2-palmitoyl-GPC (14:0/16:0)		HMDB07869	129657
1654			1-myristoyl-2-arachidonoyl-GPC (14:0/20:4)*		HMDB07883	
1663			1,2-dipalmitoyl-GPC (16:0/16:0)	D03585	HMDB00564	452110
1664			1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)*		HMDB07969	

1669			1-palmitoyl-2-stearoyl-GPC (16:0/18:0)		HMDB07970	
1673			1-palmitoyl-2-oleoyl-GPC (16:0/18:1)		HMDB07972	6436017
1678			1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)		HMDB07973	5287971
1693			1-palmitoleoyl-2-linolenoyl-GPC (16:1/18:3)*		HMDB08008	
1706		Phosphatidylcholine (PC)	1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4n6)		HMDB07982	10747814
1722			1-palmitoyl-2-docosahexaenoyl-GPC (16:0/22:6)		HMDB07991	6441886
1736			1-stearoyl-2-oleoyl-GPC (18:0/18:1)		HMDB08038	
1742			1-stearoyl-2-linoleoyl-GPC (18:0/18:2)*		HMDB08039	
1750			1,2-dilinoleoyl-GPC (18:2/18:2)		HMDB08138	5288075
1751			1-linoleoyl-2-linolenoyl-GPC (18:2/18:3)*		HMDB08141	
1758			1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)		HMDB08048	16219824
1766			1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)*		HMDB08147	
1771			1-stearoyl-2-docosahexaenoyl-GPC (18:0/22:6)		HMDB08057	
1774			1-oleoyl-2-docosahexaenoyl-GPC (18:1/22:6)*		HMDB08123	
1790			1-palmitoyl-2-oleoyl-GPE (16:0/18:1)		HMDB05320	5283496
1791			1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)		HMDB05322	9546747
1800			1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)*		HMDB05323	9546800
1804			1-palmitoyl-2-docosahexaenoyl-GPE (16:0/22:6)*		HMDB05324	9546799
1805			1-stearoyl-2-oleoyl-GPE (18:0/18:1)		HMDB08993	
1811		Phosphatidylethanolamine (PE)	1-stearoyl-2-linoleoyl-GPE (18:0/18:2)*		HMDB08994	9546749
1815			1-oleoyl-2-linoleoyl-GPE (18:1/18:2)*		HMDB05349	9546753
1824			1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)		HMDB09003	5289133
1840			1-stearoyl-2-docosahexaenoyl-GPE (18:0/22:6)*		HMDB05334	9546798
1842			1-oleoyl-2-docosahexaenoyl-GPE (18:1/22:6)*			
1858		Phosphatidylserine (PS)	1-stearoyl-2-oleoyl-GPS (18:0/18:1)		HMDB10163	9547087
1885			1-palmitoyl-2-oleoyl-GPI (16:0/18:1)*		HMDB09783	
1889			1-palmitoyl-2-linoleoyl-GPI (16:0/18:2)		HMDB09784	
1893		Phosphatidylinositol (PI)	1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4)*		HMDB09789	
1901			1-stearoyl-2-linoleoyl-GPI (18:0/18:2)		HMDB09809	
1917			1-stearoyl-2-arachidonoyl-GPI (18:0/20:4)		HMDB09815	
1927			1-palmitoyl-GPA (16:0)	C04036	HMDB00327	6419701
1931			1-linoleoyl-GPA (18:2)*		HMDB07856	
1933			1-arachidonoyl-GPA (20:4)			
1940			1-palmitoyl-GPC (16:0)		HMDB10382	86554
1941			2-palmitoyl-GPC (16:0)*		HMDB61702	15061532
1942			1-palmitoleoyl-GPC (16:1)*		HMDB10383	24779461
1943	Lipid		2-palmitoleoyl-GPC (16:1)*		HMDB10383	
1947			1-stearoyl-GPC (18:0)		HMDB10384	497299
1949			1-oleoyl-GPC (18:1)		HMDB02815	16081932
1952			1-linoleoyl-GPC (18:2)	C04100	HMDB10386	11988421
1954			1-linolenoyl-GPC (18:3)*		HMDB10388	
1968			1-arachidonoyl-GPC (20:4n6)*	C05208	HMDB10395	
1979			1-lignoceroyl-GPC (24:0)		HMDB10405	
1985			1-palmitoyl-GPE (16:0)		HMDB11503	9547069
1990		Lysophospholipid	1-stearoyl-GPE (18:0)		HMDB11130	9547068
1991			2-stearoyl-GPE (18:0)*		HMDB11129	
1992			1-oleoyl-GPE (18:1)		HMDB11506	9547071
1994			1-linoleoyl-GPE (18:2)*		HMDB11507	52925130
2001			1-arachidonoyl-GPE (20:4n6)*		HMDB11517	42607465
2009			1-stearoyl-GPS (18:0)*			9547101
2014			1-palmitoyl-GPG (16:0)*			3300276
2016			1-stearoyl-GPG (18:0)			
2018			1-oleoyl-GPG (18:1)*			
2020			1-linoleoyl-GPG (18:2)*			
2021			1-palmitoyl-GPI (16:0)		HMDB61695	
2024			1-stearoyl-GPI (18:0)		HMDB61696	
2026			1-oleoyl-GPI (18:1)*			

2028			1-linoleoyl-GPI (18:2)*			
2032			1-arachidonoyl-GPI (20:4)*		HMDB61690	
2125		Plasmalogen	1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)*		HMDB11342	
2126			1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (P-16:0/18:2)*		HMDB11343	
2127			1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0)*		HMDB11206	11146967
2128			1-(1-enyl-palmitoyl)-2-palmitoleoyl-GPC (P-16:0/16:1)*		HMDB11207	
2129			1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4)*		HMDB11352	
2131			1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1)*			
2133			1-(1-enyl-stearoyl)-2-oleoyl-GPE (P-18:0/18:1)		HMDB11375	
2134			1-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2)*		HMDB11376	
2137			1-(1-enyl-palmitoyl)-2-arachidonoyl-GPC (P-16:0/20:4)*		HMDB11220	
2138			1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)*		HMDB11211	
2141			1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4)*		HMDB05779	9547058
2153		Lysoplasmalogen	1-(1-enyl-palmitoyl)-GPC (P-16:0)*		HMDB10407	10917802
2154			1-(1-enyl-palmitoyl)-GPE (P-16:0)*			
2156			1-(1-enyl-oleoyl)-GPE (P-18:1)*			
2158			1-(1-enyl-stearoyl)-GPE (P-18:0)*			
2161		Glycerolipid Metabolism	glycerol	C00116	HMDB00131	753
2162			glycerol 3-phosphate	C00093	HMDB00126	754
2167			glycerophosphoglycerol	C03274		439964
2168		Monoacylglycerol	1-myristoylglycerol (14:0)	C01885	HMDB11561	79050
2171			1-palmitoylglycerol (16:0)		HMDB31074	14900
2172			1-palmitoleoylglycerol (16:1)*		HMDB11565	
2175			1-oleoylglycerol (18:1)		HMDB11567	5283468
2176			1-linoleoylglycerol (18:2)			5283469
2177			1-linolenoylglycerol (18:3)		HMDB11569	53480978
2180			1-dihomo-linolenylglycerol (20:3)			
2181			1-arachidonylglycerol (20:4)	C13857	HMDB11549	5282281
2184			1-docosahexaenoylglycerol (22:6)		HMDB11587	
2189			2-oleoylglycerol (18:1)		HMDB11537	5319879
2190			2-linoleoylglycerol (18:2)		HMDB11538	5365676
2191			2-arachidonoylglycerol (20:4)	C13856	HMDB04666	5282280
2220		Diacylglycerol	palmitoleoyl-linoleoyl-glycerol (16:1/18:2) [1]*		HMDB07132	
2233			oleoyl-linoleoyl-glycerol (18:1/18:2) [1]		HMDB07219	
2234			oleoyl-linoleoyl-glycerol (18:1/18:2) [2]		HMDB07219	
2238			linoleoyl-linoleoyl-glycerol (18:2/18:2) [1]*		HMDB07248	
2248			linoleoyl-arachidonoyl-glycerol (18:2/20:4) [1]*		HMDB07257	
2249			linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2]*		HMDB07257	
2276		Sphingolipid Metabolism	sphinganine-1-phosphate		HMDB01383	520
2279			N-palmitoyl-sphinganine (d18:0/16:0)		HMDB11760	5283572
2280			N-palmitoyl-sphingadienine (d18:2/16:0)*			
2289			myristoyl dihydrosphingomyelin (d18:0/14:0)*		HMDB12085	
2290			palmitoyl dihydrosphingomyelin (d18:0/16:0)*			9939965
2291			behenoyl dihydrosphingomyelin (d18:0/22:0)*		HMDB12091	
2292			palmitoyl sphingomyelin (d18:1/16:0)			9939941
2293			stearoyl sphingomyelin (d18:1/18:0)	C00550	HMDB01348	6453725
2294			behenoyl sphingomyelin (d18:1/22:0)*		HMDB12103	
2295			tricosanoyl sphingomyelin (d18:1/23:0)*		HMDB12105	
2296			lignoceroyl sphingomyelin (d18:1/24:0)			
2298			sphingomyelin (d18:1/14:0, d16:1/16:0)*		HMDB12097	11433862
2299			sphingomyelin (d18:2/14:0, d18:1/14:1)*			
2300			sphingomyelin (d17:1/16:0, d18:1/15:0, d16:1/17:0)*			
2301			sphingomyelin (d18:2/16:0, d18:1/16:1)*			
2302			sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)			
2303			sphingomyelin (d18:1/18:1, d18:2/18:0)		HMDB12101	6443882
2304			sphingomyelin (d18:1/20:0, d16:1/22:0)*		HMDB12102	
2305			sphingomyelin (d18:1/20:1, d18:2/20:0)*			
2306			sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*			

2307			sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)*		HMDB12104	
2308			sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)*			
2309			sphingomyelin (d18:1/24:1, d18:2/24:0)*		HMDB12107	
2310			sphingomyelin (d18:2/24:1, d18:1/24:2)*			
2313			sphingosine	C00319	HMDB00252	5353955
2314			sphingosine 1-phosphate	C06124	HMDB00277	5283560
2317			sphingomyelin (d18:2/23:1)*			
2318			sphingomyelin (d18:2/21:0, d16:2/23:0)*			
2319			sphingomyelin (d18:1/20:2, d18:2/20:1, d16:1/22:2)*			
2320			sphingomyelin (d18:2/24:2)*			
2323			sphingomyelin (d18:1/25:0, d19:0/24:1, d20:1/23:0, d19:1/24:0)*			
2324			sphingomyelin (d18:1/22:2, d18:2/22:1, d16:1/24:2)*			
2325			sphingomyelin (d18:0/20:0, d16:0/22:0)*			
2326			sphingomyelin (d18:0/18:0, d19:0/17:0)*		HMDB12087	
2327			sphingomyelin (d17:2/16:0, d18:2/15:0)*			
2328			sphingomyelin (d18:2/18:1)*			
2329			sphingomyelin (d18:1/19:0, d19:1/18:0)*			
2376		Ceramides	N-palmitoyl-sphingosine (d18:1/16:0)		HMDB04949	5283564
2378			N-stearoyl-sphingosine (d18:1/18:0)*		HMDB04950	5283565
2382			ceramide (d18:1/14:0, d16:1/16:0)*			
2385			ceramide (d18:2/24:1, d18:1/24:2)*			
2386			glycosyl-N-palmitoyl-sphingosine (d18:1/16:0)			
2387			glycosyl-N-stearoyl-sphingosine (d18:1/18:0)			
2390			glycosyl-N-behenoyl-sphingadienine (d18:2/22:0)*			
2396			lactosyl-N-palmitoyl-sphingosine (d18:1/16:0)			
2398			lactosyl-N-nervonoyl-sphingosine (d18:1/24:1)*			
2401			glycosyl ceramide (d18:1/20:0, d16:1/22:0)*			
2403			glycosyl ceramide (d18:2/24:1, d18:1/24:2)*			
2410		Mevalonate Metabolism	3-hydroxy-3-methylglutarate	C03761	HMDB00355	1662
2425		Sterol	cholesterol	C00187	HMDB00067	11025495
2435			7-alpha-hydroxy-3-oxo-4-cholestenoate (7-Hoca)	C17337	HMDB12458	3081085
2438			3beta-hydroxy-5-cholestenoate	C17333		165511
2470		Pregnenolone Steroids	pregnenolone sulfate		HMDB00774	105074
2474			17alpha-hydroxypregnenolone 3-sulfate		HMDB00416	152971
2475			17alpha-hydroxypregnanolone glucuronide			
2477			21-hydroxypregnenolone monosulfate (1)			174681
2480			21-hydroxypregnenolone disulfate			134595
2491		Progestin Steroids	5alpha-pregnan-3beta-ol,20-one sulfate			
2492			5alpha-pregnan-3beta,20beta-diol monosulfate (1)			
2495			5alpha-pregnan-3beta,20alpha-diol monosulfate (2)			
2500			5alpha-pregnan-3beta,20alpha-diol disulfate			
2503			pregnanediol-3-glucuronide		HMDB10318	123796
2508			pregnanolone/allopregnanolone sulfate			
2509			pregnen-diol disulfate C21H34O8S2*			
2510			pregn steroid monosulfate C21H34O5S*			
2520		Corticosteroids	corticosterone	C02140	HMDB01547	5753
2525			cortisol	C00735	HMDB00063	5754
2527			cortisone	C00762	HMDB02802	222786
2538			11-ketoetiocholanolone glucuronide			
2539			dehydroisoandrosterone sulfate (DHEA-S)	C04555	HMDB01032	12594
2540			16a-hydroxy DHEA 3-sulfate			
2542			epiandrosterone sulfate			9929317
2550			androsterone sulfate		HMDB02759	159663
2553			etiocholanolone glucuronide		HMDB04484	270605
2554			5alpha-androstan-3alpha,17alpha-diol monosulfate			
2556			androstenediol (3beta,17beta) monosulfate (1)		HMDB03818	13847309
2557			androstenediol (3beta,17beta) monosulfate (2)			

2559		Androgenic Steroids	androstenediol (3beta,17beta) disulfate (1)	C04295	HMDB03818	87120982
2560			androstenediol (3beta,17beta) disulfate (2)	C04295	HMDB03818	87120982
2563			androstenediol (3alpha, 17alpha) monosulfate (2)			
2564			androstenediol (3alpha, 17alpha) monosulfate (3)			
2566			testosterone sulfate		HMDB02833	119207
2573			5alpha-androstan-3alpha,17beta-diol monosulfate (1)			
2574			5alpha-androstan-3alpha,17beta-diol monosulfate (2)			
2575			5alpha-androstan-3alpha,17beta-diol disulfate			
2576			5alpha-androstan-3alpha,17beta-diol 17-glucuronide			
2579			5alpha-androstan-3beta,17beta-diol monosulfate (1)			
2580			5alpha-androstan-3beta,17beta-diol monosulfate (2)			
2581			5alpha-androstan-3beta,17beta-diol disulfate	C12525	HMDB00493	242332
2584			5alpha-androstan-3beta,17alpha-diol disulfate			
2588			andro steroid monosulfate C19H28O6S (1)*	C04555	HMDB02759	
2611		Primary Bile Acid Metabolism	cholate	C00695	HMDB00619	221493
2612			glycocholate	C01921	HMDB00138	10140
2613			taurocholate	C05122	HMDB00036	6675
2614			chenodeoxycholate	C02528	HMDB00518	10133
2615			glycochenodeoxycholate	C05466	HMDB00637	12544
2616			taurochenodeoxycholate	C05465	HMDB00951	387316
2620			tauro-beta-muricholate		HMDB00932	168408
2623			glycochenodeoxycholate glucuronide (1)			
2625			glycochenodeoxycholate sulfate			
2626			glycocholate glucuronide (1)			
2628		Secondary Bile Acid Metabolism	deoxycholate	C04483	HMDB00626	222528
2630			glycodeoxycholate	C05464	HMDB00631	3035026
2631			taurodeoxycholate	C05463	HMDB00896	2733768
2635			glycolithocholate	C15557	HMDB00698	115245
2636			glycolithocholate sulfate*	C11301	HMDB02639	72222
2638			taurolithocholate 3-sulfate	C03642	HMDB02580	440071
2640			ursodeoxycholate	C07880	HMDB00946	31401
2641			isoursodeoxycholate	C17662	HMDB00686	127601
2642			glycoursodeoxycholate		HMDB00708	12310288
2643			tauroursodeoxycholate		HMDB00874	9848818
2649			hyocholate	C17649	HMDB00760	92805
2650			glycohyocholate			
2659			glycocholenate sulfate*			
2660			taurocholenate sulfate			
2663			3b-hydroxy-5-cholenoic acid		HMDB00308	92997
2664			glycodeoxycholate sulfate			
2666			ursodeoxycholate sulfate (1)			
2668			glycodeoxycholate glucuronide (1)			
3235		Purine Metabolism, (Hypo)Xanthine/Inosine containing	inosine	C00294	HMDB00195	6021
3236			hypoxanthine	C00262	HMDB00157	790
3237			xanthine	C00385	HMDB00292	1188
3239			xanthosine	C01762	HMDB00299	64959
3242			N1-methylinosine		HMDB02721	65095
3245			urate	C00366	HMDB00289	1175
3246			allantoin	C02350	HMDB00462	204
3252		Purine Metabolism, Adenine containing	adenosine 5'-monophosphate (AMP)	C00020	HMDB00045	6083
3255			adenosine 3',5'-cyclic monophosphate (cAMP)	C00575	HMDB00058	6076
3259			adenosine	C00212	HMDB00050	60961
3260			adenine	C00147	HMDB00034	190
3265			N1-methyladenosine	C02494	HMDB03331	27476
3275			N6-carbamoylthreonyladenosine		HMDB41623	161466
3284			N6-succinyladenosine		HMDB00912	165243
3292		Purine Metabolism, Guanine containing	guanosine	C00387	HMDB00133	6802
3295			7-methylguanine	C02242	HMDB00897	11361

3301			N2,N2-dimethylguanosine		HMDB04824	92919
3312	Nucleotide	Pyrimidine Metabolism, Orotate containing	dihydroorotate	C00337	HMDB03349	648
3313			orotate	C00295	HMDB00226	967
3315			orotidine		HMDB00788	92751
3325		Pyrimidine Metabolism, Uracil containing	uridine	C00299	HMDB00296	6029
3326			uracil	C00106	HMDB00300	1174
3327			pseudouridine	C02067	HMDB00767	15047
3328			2'-O-methyluridine			102212
3329			5-methyluridine (ribothymidine)		HMDB00884	445408
3340			2'-deoxyuridine	C00526	HMDB00012	13712
3343			3-ureidopropionate	C02642	HMDB00026	111
3344			beta-alanine	C00099	HMDB00056	239
3345			N-acetyl-beta-alanine	C01073		76406
3353		Pyrimidine Metabolism, Cytidine containing	cytidine	C00475	HMDB00089	6175
3354			cytosine	C00380	HMDB00630	597
3355			3-methylcytidine			159649
3357			N4-acetylcytidine		HMDB05923	107461
3364			2'-O-methylcytidine			150971
3375		Pyrimidine Metabolism, Thymine containing	5,6-dihydrothymine	C00906	HMDB00079	93556
3377			3-aminoisobutyrate	C05145	HMDB03911	64956
3381	Cofactors/Vitamins	Nicotinate and Nicotinamide Metabolism	quinolinate	C03722	HMDB00232	1066
3383			nicotinate ribonucleoside	C05841	HMDB06809	161234
3385			nicotinamide	C00153	HMDB01406	936
3397			1-methylnicotinamide	C02918	HMDB00699	10129985
3402			trigonelline (N'-methylnicotinate)	C01004	HMDB00875	5570
3403			nicotinurate	C05380	HMDB03269	68499
3404			N1-Methyl-2-pyridone-5-carboxamide	C05842	HMDB04193	69698
3412		Pantothenate and CoA Metabolism	pantothenate	C00864	HMDB00210	6613
3423		Ascorbate and Aldarate Metabolism	ascorbate (Vitamin C)	C00072	HMDB00044	
3425			threonate	C01620	HMDB00943	151152
3428			oxalate (ethanedioate)	C00209	HMDB02329	971
3429			gulonate*	C00257	HMDB03290	9794176
3431		Tocopherol Metabolism	alpha-tocopherol	C02477	HMDB01893	14985
3439			gamma-CEHC		HMDB01931	133098
3440			gamma-CEHC glucuronide*			
3441			alpha-CEHC glucuronide*			
3442			alpha-CEHC sulfate			
3443			alpha-CEHC		HMDB01518	9943542
3445			gamma-tocopherol/beta-tocopherol			
3449		Folate Metabolism	folate	C00504	HMDB00121	6037
3451			5-methyltetrahydrofolate (5MeTHF)	C00440	HMDB01396	146
3467		Hemoglobin and Porphyrin Metabolism	heme	C00032	HMDB03178	26945
3468			bilirubin (Z,Z)	C00486	HMDB00054	5280352
3469			bilirubin (E,E)*			5315454
3470			bilirubin (E,Z or Z,E)*		HMDB00488	5799469
3471			biliverdin	C00500	HMDB01008	5353439
3472			I-urobilinogen	C05790	HMDB04157	26818
3488		Vitamin A Metabolism	retinol (Vitamin A)	C00473	HMDB00305	445354
3490			carotene diol (1)			
3491			carotene diol (2)			
3492			carotene diol (3)			
3494			beta-cryptoxanthin		HMDB33844	6384256
3507		Vitamin B6 Metabolism	pyridoxal	C00250	HMDB01545	1050
3508			pyridoxate	C00847	HMDB00017	6723
3510			hippurate	C01586	HMDB00714	464
3514			2-hydroxyhippurate (salicylurate)	C07588	HMDB00840	10253
3515			3-hydroxyhippurate		HMDB06116	450268

3516			4-hydroxyhippurate		HMDB13678	151012
3522			benzoate	C00180	HMDB01870	243
3540			catechol sulfate		HMDB59724	3083879
3542			O-methylcatechol sulfate		HMDB60013	22473
3543			3-methyl catechol sulfate (1)			
3544			3-methyl catechol sulfate (2)			
3545			4-methylcatechol sulfate			
3555		Benzoate Metabolism	4-ethylphenylsulfate	C13637		
3556			4-vinylphenol sulfate	C05627	HMDB04072	6426766
3568			3-methoxycatechol sulfate (1)			
3569			3-methoxycatechol sulfate (2)			
3570			methyl-4-hydroxybenzoate sulfate			
3572			propyl 4-hydroxybenzoate sulfate			
3577			p-cresol sulfate		HMDB11635	4615423
3584			o-cresol sulfate			11615528
3588			3-(3-hydroxyphenyl)propionate sulfate			187488
3591			3-(3-hydroxyphenyl)propionate	C11457	HMDB00375	91
3592			3-(4-hydroxyphenyl)propionate	C01744	HMDB02199	10394
3593			3-phenylpropionate (hydrocinnamate)	C05629	HMDB00764	107
3595		Xanthine Metabolism	caffeine	C07481	HMDB01847	2519
3596			paraxanthine	C13747	HMDB01860	4687
3597			theobromine	C07480	HMDB02825	5429
3598			theophylline	C07130	HMDB01889	2153
3599			1-methylurate	C16359	HMDB03099	69726
3600			7-methylurate	C16355	HMDB11107	69160
3601			1,3-dimethylurate		HMDB01857	70346
3602			1,7-dimethylurate	C16356	HMDB11103	91611
3603			3,7-dimethylurate	C16360	HMDB01982	83126
3604			1,3,7-trimethylurate	C16361	HMDB02123	79437
3605			1-methylxanthine	C16358	HMDB10738	80220
3606			3-methylxanthine	C16357	HMDB01886	70639
3607			7-methylxanthine	C16353	HMDB01991	68374
3608			5-acetylamino-6-amino-3-methyluracil	C16366	HMDB04400	88299
3609			5-acetylamino-6-formylamino-3-methyluracil	C16365	HMDB11105	108214
3611			caffeic acid sulfate		HMDB41708	
3612		Tobacco Metabolite	cotinine		HMDB01046	854019
3613			hydroxycotinine		HMDB01390	10219774
3614			cotinine N-oxide		HMDB01411	9815514
3616			3-hydroxycotinine glucuronide		HMDB01204	183115
3627			2-piperidinone		HMDB11749	12665
3665			sucralose	C12285	HMDB31554	71485
3710			2,3-dihydroxyisovalerate	C04039	HMDB12141	677
3711			2,3-dihydroxypyridine			28115
3717			2-isopropylmalate	C02504	HMDB00402	77
3736			betonicine	C08269	HMDB29412	164642
3740			gluconate	C00257	HMDB00625	10690
3749			alliin	C08265	HMDB33592	87310
3750			N-acetylalliin			
3781			cinnamoylglycine		HMDB11621	709625
3803			dihydroferulic acid			14340
3812			ergothioneine	C05570	HMDB03045	3032311
3814			erythritol	C00503	HMDB02994	222285
3817			ferulic acid 4-sulfate		HMDB29200	6305574
3838			homostachydrine*	C08283	HMDB33433	441447
3842			indolin-2-one	C12312		321710
3864			methyl indole-3-acetate		HMDB29738	74706
3867			N-(2-furoyl)glycine		HMDB00439	21863
3868			N-oxalyl glycine (NOG)			3080614

3871		Food Component/Plant	naringenin 7-glucuronide			
3893			piperine	C03882	HMDB29377	638024
3903			quinate	C00296	HMDB03072	6508
3908			saccharin	D01085	HMDB29723	5143
3910			acesulfame		HMDB33585	36573
3911			S-allylcysteine		HMDB34323	98280
3928			stachydrine	C10172	HMDB04827	115244
3935			tartarate	C00898	HMDB00956	444305
3936			theanine	C01047	HMDB34365	439378
3938			thymol sulfate	C09908	HMDB01878	
3951			4-allylphenol sulfate			
3952			methyl glucopyranoside (alpha + beta)			
3979			4-vinylguaiacol sulfate			
3980			pyrraline		HMDB33143	122228
3981			umbelliferone sulfate			129659
3982			daidzein sulfate (2)			
3984			eugenol sulfate			180632
3988			2-keto-3-deoxy-gluconate	C00204	HMDB01353	161227
3989			3-hydroxycinnamate sulfate			6443141
4010			3,4-methyleneheptanoate			
4055		Bacterial/Fungal	tartronate (hydroxymalonate)	C02287	HMDB35227	45
4096			azithromycin	C06838	HMDB14352	2269
4100			2-hydroxyacetaminophen sulfate*			86290013
4101			2-methoxyacetaminophen sulfate*			86290014
4102			3-(cystein-S-yl)acetaminophen*			5233914
4103	Xenobiotics		3-(N-acetyl-L-cystein-S-yl) acetaminophen			83967
4104			4-acetaminophen sulfate	C06804	HMDB59911	83939
4106			4-acetamidophenol	C06804	HMDB01859	1983
4107			4-acetamidophenylglucuronide		HMDB10316	83944
4108			2-methoxyacetaminophen glucuronide*			14367271
4109			salicyluric glucuronide*			
4110			ibuprofen acyl glucuronide			163959
4111			ibuprofen	D00126	HMDB01925	3672
4112			2-hydroxyibuprofen		HMDB60920	10443535
4114			carboxyibuprofen		HMDB60564	10444113
4118			1-hydroxy-2-naphthalenecarboxylate	C03203		6844
4126			4-acetylphenol sulfate			4684006
4139			allopurinol			2094
4140			allopurinol riboside			
4142			amoxicillin	C06827	HMDB15193	2171
4149			atenolol	D00235	HMDB01924	2249
4164			carbamazepine	D00252	HMDB14704	2554
4165			carbamazepine 10,11-epoxide*	C07496	HMDB60658	2555
4166			carbamazepine glucuronide*			
4167			2-hydroxycarbamazepine	C16601	HMDB60651	129274
4176			ciprofloxacin	C05349		4011971;2764
4184			4-hydroxycoumarin	C20414		54682930
4187			desmethylnaproxen		HMDB13989	13393711
4188			desmethylnaproxen sulfate			184679
4193			diltiazem	C06958	HMDB14487	39186
4194			diphenhydramine	C06960	HMDB01927	3100
4196			doxycycline			
4200			lisinopril			3937
4203			escitalopram	D07913	HMDB05028	146570
4207			fexofenadine	C06999	HMDB05030	3348
4211			fluoxetine	D00326		3386
4213		Drug	furosemide	D00331	HMDB01933	3440

4218			lamotrigine	D00354		3878
4221			hydrochlorothiazide	C07041	HMDB01928	3639
4223			hydroquinone sulfate	C00530	HMDB02434	161220
4224			hydroxybupropion		HMDB12235	446
4252			metformin	C07151	HMDB01921	4091
4257			metoprolol	D02358	HMDB01932	4171
4258			alpha-hydroxymetoprolol		HMDB60994	114962
4259			metoprolol acid metabolite*			62936
4267			naproxen	C01517	HMDB01923	156391
4274			norfluoxetine		HMDB60551	4541
4275			ofloxacin	C07321	HMDB01929	4583
4277			omeprazole	C07324	HMDB01913	4594
4278			dexlansoprazole	D08903		9578005
4282			oxypurinol	D02365	HMDB00786	4644
4283			pantoprazole	C11806	HMDB05017	4679
4298			pseudoephedrine	C02765	HMDB01943	7028
4303			ranitidine	D00422	HMDB01930	3001055
4307			rosuvastatin	D01915	HMDB15230	446157
4309			salicylate	C00805	HMDB01895	338
4322			ticlopidine	C07140	HMDB14353	5472
4324			trazadone		HMDB14794	5533
4326			triamterene	D00386	HMDB01940	5546
4335			verapamil	D02356	HMDB01850	2520
4338			warfarin	C01541	HMDB01935	
4351			o-hydroxyatorvastatin			9808225
4358			2-acetamidophenol sulfate			181671
4360			valsartan			5650
4366			tramadol	C07153	HMDB14339	33741
4367			O-desmethyltramadol glucuronide			
4378			pregabalin			4715169
4383			O-desmethyltramadol		HMDB60997	130829
4385			olmesartan	D05246	HMDB14420	158781
4387			guaifenesin	D00337	HMDB04998	3516
4390			cetirizine	C07778	HMDB05032	2678
4391			candesartan		HMDB14934	2541
4473			sulfate*	C00059	HMDB01448	1118
4476			O-sulfo-L-tyrosine			514186
4480			ethyl glucuronide		HMDB10325	152226
4492			3-acetylphenol sulfate			
4494			2-aminophenol sulfate		HMDB61116	181670
4538			dimethyl sulfone	C11142	HMDB04983	6213
4539			dimethyl sulfoxide (DMSO)	C11143	HMDB02151	679
4540			ectoine	C06231		126041
4551			lanthionine			98504
4568		Chemical	perfluorooctanesulfonic acid (PFOS)	C18142	HMDB59586	74483
4577			succinimide	C07273		11439
4589			4-hydroxychlorothalonil			34217
4593			1,2,3-benzenetriol sulfate (2)			
4594			2-methoxyresorcinol sulfate			
4595			3-hydroxypyridine sulfate			
4596			1,2,3-benzenetriol sulfate (1)			
4606			6-hydroxyindole sulfate			
4609			4-acetamidobenzoate	D03836		19266
4610			thioproline			93176

Appendix 3. Serum metabolites with most highly statistically significant p-values.

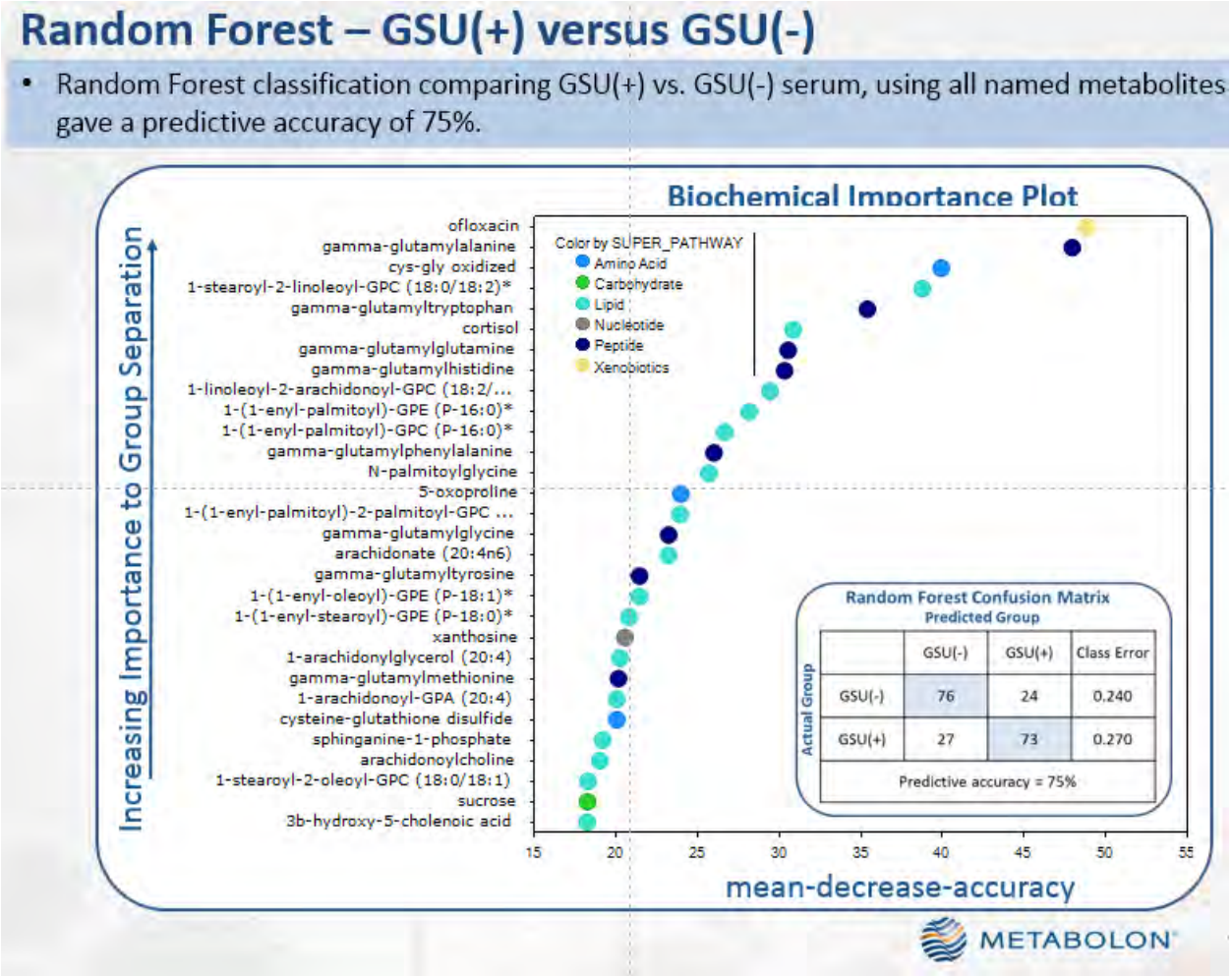
Metabolites with lowest p-values for GSU(+) vs. GSU(-) comparison

Biochemical Name	ratio							p-value
	GSU(+) All GSU(-) All	GSU(+) BMI <25 GSU(-) BMI <25	GSU(+) BMI 25-29.9 GSU(-) BMI 25-29.9	GSU(+) BMI ≥30 GSU(-) BMI ≥30	GSU(+) Age <60 GSU(-) Age <60	GSU(+) Age 60-69 GSU(-) Age 60-69	GSU(+) Age ≥70 GSU(-) Age ≥70	GSU(+) All GSU(-) All
gamma-glutamylalanine	0.69	0.64	0.72	0.73	0.54	0.73	0.73	5.08E-08
gamma-glutamylhistidine	0.81	0.78	0.80	0.87	0.71	0.82	0.86	9.05E-07
gamma-glutamyltryptophan	0.71	0.74	0.69	0.81	0.59	0.74	0.74	4.22E-06
1-(1-enyl-palmitoyl)-GPE (P-16:0)*	1.52	1.57	1.55	1.46	1.19	1.51	1.57	5.73E-06
1-(1-enyl-palmitoyl)-GPC (P-16:0)*	1.50	1.53	1.59	1.16	0.97	1.43	1.79	7.85E-06
gamma-glutamylphenylalanine	0.81	0.83	0.79	0.97	0.76	0.79	0.83	9.97E-06
cys-gly, oxidized	0.63	0.63	0.66	0.61	0.48	0.69	0.93	2.68E-05
1-(1-enyl-palmitoyl)-2-palmitoleoyl-GPC (P-16:0/16:1)*	1.18	1.21	1.21	0.94	1.02	1.16	1.15	4.66E-05
1-(1-enyl-stearoyl)-GPE (P-18:0)*	1.55	1.51	1.69	1.33	1.10	1.51	1.59	4.73E-05
1-stearoyl-2-linoleoyl-GPC (18:0/18:2)*	0.92	0.90	0.92	0.94	0.95	0.93	0.95	5.33E-05
gamma-glutamylleucine	0.81	0.88	0.79	0.89	0.81	0.83	0.82	5.90E-05
3b-hydroxy-5-cholenoic acid	1.40	1.50	1.56	1.19	1.23	1.42	2.07	9.45E-05
N-oleoylserine	1.19	1.26	1.18	1.10	1.09	1.19	1.21	1.00E-04
1-arachidonoyl-GPA (20:4)	1.60	1.50	1.80	1.45	1.46	1.81	1.35	1.00E-04
1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0)*	1.12	1.08	1.17	1.00	1.12	1.11	1.10	1.00E-04
gamma-glutamyltyrosine	0.79	0.84	0.76	0.95	0.64	0.82	0.76	2.00E-04
gamma-glutamylvaline	0.81	0.82	0.81	0.87	0.77	0.76	0.88	2.00E-04
1-arachidonylglycerol (20:4)	1.34	1.52	1.21	1.39	1.33	1.36	1.49	2.00E-04
gamma-glutamylglutamine	0.82	0.83	0.82	0.90	0.58	0.86	0.93	3.00E-04
gamma-glutamylglycine	0.81	0.77	0.81	0.84	0.55	0.83	0.79	3.00E-04
1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	0.89	0.89	0.89	0.84	0.80	0.95	0.90	3.00E-04
gamma-glutamylmethionine	0.79	0.84	0.74	0.94	0.60	0.83	0.75	4.00E-04
arachidonate (20:4n8)	1.21	1.29	1.15	1.34	1.62	1.23	1.30	4.00E-04
N-palmitoylglycine	1.15	1.18	1.13	1.13	1.27	1.15	1.13	4.00E-04
1,2-dilinoleoyl-GPC (18:2/18:2)	0.84	0.79	0.84	0.80	0.73	0.86	0.95	5.00E-04

Values represent ratio of metabolite expression in cases vs. controls. Values in dark green are significant decreased, light green .05<p<.10; values in dark red are significantly decreased, pink .05<p<.10. All p-values represent nominal p-values; no adjustment for multiple comparisons or false-discovery.

* Abbreviations: GSU(+), Gleason score upgrade (cases); GSU(-), Gleason score not upgraded (controls); GPC, glycerophosphocholine; GPE, glycerophoshoethanolamine; GPA, glycerophosphatidic acid.

Appendix 4. Random forest plot of serum metabolites significantly associated with Gleason score upgrade cases (GSU+) compared to no Gleason score upgrade controls (GSU-).



* Abbreviations: GSU(+), Gleason score upgrade (cases); GSU(-), Gleason score not upgraded (controls); GPC, glycerophosphocholine; GPE, glycerophoshoethanolamine; GPA, glycerophosphatidic acid.

Higher values of "mean-decrease-accuracy" indicate stronger associations with outcome.

Johns Hopkins University JHOP-0802-16VW

Gleason score stratification in prostate cancer: urine

Biochemicals profiles in this study, by Super Pathway & Sub-pathway

Pathway Sort Order	Super Pathway	Sub Pathway	Biochemical Name	KEGG	HMDB	PubChem
1		Glycine, Serine and Threonine Metabolism	glycine	C00037	HMDB00123	750
2			N-acetylglycine		HMDB00532	10972
4			sarcosine	C00213	HMDB00271	1088
5			dimethylglycine	C01026	HMDB00092	673
6			betaine	C00719	HMDB00043	247
9			serine	C00065	HMDB00187	5951
10			N-acetylserine		HMDB02931	65249
16			threonine	C00188	HMDB00167	6288
17			N-acetylthreonine			152204
18			allo-threonine	C05519	HMDB04041	99289
28		Alanine and Aspartate Metabolism	alanine	C00041	HMDB00161	5950
30			N-acetylalanine	C02847	HMDB00766	88064
33			N-carbamoylalanine			426409
34			aspartate	C00049	HMDB00191	5960
35			N-acetylaspartate (NAA)	C01042	HMDB00812	65065
38			asparagine	C00152	HMDB00168	6267
39			N-acetylasparagine		HMDB06028	99715
40		Glutamate Metabolism	glutamate	C00025	HMDB00148	611
41			glutamine	C00064	HMDB00641	5961
42			N-acetylglutamate	C00624	HMDB01138	70914
43			N-acetylglutamine	C02716	HMDB06029	182230
44			N-methylglutamate	C01046		439377
45			4-hydroxyglutamate	C03079	HMDB01344	439902
47			gamma-carboxyglutamate		HMDB41900	40772
49			pyroglutamine*			134508
50			N-acetyl-aspartyl-glutamate (NAAG)	C12270	HMDB01067	5255
51			beta-citrylglutamate	C20775		72715786
53			carboxyethyl-GABA		HMDB02201	2572
54			N-methyl-GABA	C15987		70703
55			2-pyrrolidinone		HMDB02039	12025
57			S-1-pyrroline-5-carboxylate	C04322	HMDB01301	1196
58			citramalate	C00815	HMDB00426	1081
61			succinylglutamine			
62		Histidine Metabolism	histidine	C00135	HMDB00177	6274
63			1-methylhistidine	C01152	HMDB00001	92105
64			3-methylhistidine	C01152	HMDB00479	64969
65			N-acetylhistidine	C02997	HMDB32055	75619
66			N-acetyl-3-methylhistidine*			193270
67			N-acetyl-1-methylhistidine*			193270
68			hydantoin-5-propionic acid	C05565	HMDB01212	782
69			trans-urocanate	C00785	HMDB00301	736715
70			cis-urocanate		HMDB34174	1549103
71			imidazole propionate		HMDB02271	70630
72			formiminoglutamate	C00439	HMDB00854	439233
73			imidazole lactate	C05568	HMDB02320	440129
74			carnosine	C00386	HMDB00033	439224
75			homocarnosine	C00884	HMDB00745	10243361
76			N-acetylcarnosine		HMDB12881	9903482
77			anserine	C01262	HMDB00194	112072
80			1-methylimidazoleacetate	C05828	HMDB02820	75810
81			4-imidazoleacetate	C02835	HMDB02024	96215

85			N-acetylhistamine	C05135	HMDB13253	69602
87		Lysine Metabolism	lysine	C00047	HMDB00182	5962
88			N2-acetyllysine	C12989	HMDB00446	92907
89			N6-acetyllysine	C02727	HMDB00206	92832
91			N2,N6-diacetyllysine			91827
93			N6-carboxyethyllysine			
94			N6,N6,N6-trimethyllysine	C03793	HMDB01325	440120
95			5-hydroxylysine	C16741	HMDB00450	1029
96			5-(galactosylhydroxy)-L-lysine			
98			2-aminoadipate	C00956	HMDB00510	469
100			2-oxoadipate	C00322	HMDB00225	71
102			glutarylcarnitine (C5-DC)		HMDB13130	71464488
106			pipecolate	C00408	HMDB00070	849
107			6-oxopiperidine-2-carboxylate		HMDB61705	3014237
109			N-acetyl-cadaverine		HMDB02284	189087
111			N-trimethyl 5-aminovalerate			
112		Phenylalanine Metabolism	phenylalanine	C00079	HMDB00159	6140
113			N-acetylphenylalanine	C03519	HMDB00512	74839
117			phenyllactate (PLA)	C05607	HMDB00779	3848
120			phenethylamine	C05332	HMDB02017	1001
122			4-hydroxyphenylacetate	C00642	HMDB00020	127
123			3-hydroxyphenylacetate	C05593	HMDB00440	12122
128		Tyrosine Metabolism	tyrosine	C00082	HMDB00158	6057
129			N-acetyltyrosine		HMDB00866	68310
133			tyramine	C00483	HMDB00306	5610
134			m-tyramine		HMDB04989	11492
136			4-hydroxyphenylpyruvate	C01179	HMDB00707	979
138			4-hydroxyphenylacetatoylcarnitine			
139			3-(4-hydroxyphenyl)lactate	C03672	HMDB00755	9378
143			phenol sulfate	C02180	HMDB60015	74426
145			dihydoxyphenylalanine (L-DOPA)	C00355	HMDB00181	6047
147			dopamine	C03758	HMDB00073	681
153			vanillactate		HMDB00913	160637
154			vanillylmandelate (VMA)	C05584	HMDB00291	1245
156			3-methoxytyrosine		HMDB01434	1670
157			3-methoxytyramine	C05587	HMDB00022	1669
158			3-methoxytyramine sulfate			
159			3,4-dihydroxyphenylacetate	C01161	HMDB01336	547
162			homovanillate (HVA)	C05582	HMDB00118	1738
171			gentisate	C00628	HMDB00152	3469
172			5-hydroxymethyl-2-furoic acid	C20448	HMDB02432	80642
173			2-hydroxyphenylacetate	C05852	HMDB00669	11970
176			dopamine 4-sulfate	C13691	HMDB04148	123932
177			dopamine 3-O-sulfate	C13690	HMDB06275	122136
178			p-cresol-glucuronide*		HMDB11686	154035
179			tyramine O-sulfate		HMDB06409	153005
181			vanillic alcohol sulfate			
184			3,4-dihydroxyphenylacetate sulfate			193283
188			3-hydroxyphenylacetate sulfate			
189			3-hydroxyphenylacetatoylcarnitine			
191			catechol glucuronide			75124209
200	Amino Acid		tryptophan	C00078	HMDB00929	6305
201			N-acetyltryptophan	C03137	HMDB13713	700653
207			C-glycosyltryptophan			10981970
209			tryptophan betaine	C09213	HMDB61115	442106
211			kynurenine	C00328	HMDB00684	161166
213			N-acetylkynurenine (2)			
215			kynurenate	C01717	HMDB00715	3845
217			N-formylanthranilic acid	C05653	HMDB04089	101399
218			anthranilate	C00108	HMDB01123	227
220			3-hydroxykynurenine	C02794	HMDB00732	89

221		Tryptophan Metabolism	xanthurenate	C02470	HMDB00881	5699
222			3-hydroxyanthranilate	C00632	HMDB01476	86
223			picolinate	C10164	HMDB02243	1018
224			serotonin	C00780	HMDB00259	5202
227			5-hydroxyindoleacetate	C05635	HMDB00763	1826
229			tryptamine	C00398	HMDB00303	1150
231			indolelactate	C02043	HMDB00671	92904
232			indoleacetate	C00954	HMDB00197	802
235			indolepropionylglycine			7677842
236			indoleacetylglutamine		HMDB13240	25200879
239			indole-3-carboxylic acid	C19837	HMDB03320	69867
241			indoleacetylglycine			446640
242			5-hydroxyindole sulfate			
243			7-hydroxyindole sulfate			
244			3-indoxyl sulfate		HMDB00682	10258
246			5-bromotryptophan			96735
247		Leucine, Isoleucine and Valine Metabolism	leucine	C00123	HMDB00687	6106
248			N-acetylleucine	C02710	HMDB11756	70912
249			N-methylleucine			2777993
250			4-methyl-2-oxopentanoate	C00233	HMDB00695	70
257			isovalerylglycine		HMDB00678	546304
259			isovalerylglutamine			
264			3-methylcrotonylglycine		HMDB00459	169485
265			beta-hydroxyisovalerate		HMDB00754	69362
268			3-methylglutaconate		HMDB00522	1551553
272			3-methylglutarylcarnitine (2)		HMDB00552	128145
281			isoleucine	C00407	HMDB00172	6306
283			N-acetylisoleucine		HMDB61684	2802421
284			3-methyl-2-oxovalerate	C00671	HMDB03736	47
285			alpha-hydroxyisovalerate		HMDB00407	99823
287			2-methylbutyrylcarnitine (C5)		HMDB00378	6426901
288			2-methylbutyrylglycine		HMDB00339	193872
289			tiglylcarnitine (C5:1-DC)		HMDB02366	22833596
290			tigloylglycine		HMDB00959	6441567
291			3-hydroxy-2-methylbutyrate		HMDB00354	160471
292			3-hydroxy-2-ethylpropionate		HMDB00396	188979
294			ethylmalonate		HMDB00622	11756
295			methylsuccinate		HMDB01844	10349
296			methylsuccinoylcarnitine (1)			
301			valine	C00183	HMDB00883	6287
302			N-acetylvaline		HMDB11757	66789
304			3-methyl-2-oxobutyrate	C00141	HMDB00019	49
307			isobutyrylcarnitine (C4)		HMDB00736	168379
308			isobutyrylglycine		HMDB00730	10855600
309			3-hydroxyisobutyrate	C06001	HMDB00336	87
310			2,3-dihydroxy-2-methylbutyrate		HMDB29576	301941
314		Methionine, Cysteine, SAM and Taurine Metabolism	N-acetylmethionine	C02712	HMDB11745	448580
315			N-formylmethionine	C03145	HMDB01015	439750
317			methionine sulfone			69961
318			methionine sulfoxide	C02989	HMDB02005	158980
319			N-acetylmethionine sulfoxide			193368
324			S-adenosylhomocysteine (SAH)	C00021	HMDB00939	439155
328			cystathionine	C02291	HMDB00099	439258
330			cysteine	C00097	HMDB00574	5862
331			N-acetylcysteine	C06809	HMDB01890	12035
333			S-methylcysteine sulfoxide		HMDB29432	82142
335			cysteine s-sulfate	C05824	HMDB00731	115015
336			cystine	C00491	HMDB00192	67678
341			hypotaurine	C00519	HMDB00965	107812
342			taurine	C00245	HMDB00251	1123
343			N-acetyltaurine			159864

344			N-methyltaurine			7882
345			taurocyamine	C01959	HMDB03584	68340
349		Urea cycle; Arginine and Proline Metabolism	arginine	C00062	HMDB00517	232
350			argininosuccinate	C03406	HMDB00052	828
351			urea	C00086	HMDB00294	1176
352			methylurea	C16363		11719
353			ornithine	C00077	HMDB03374	6262
356			2-oxoarginine*	C03771	HMDB04225	558
357			citrulline	C00327	HMDB00904	9750
358			homoarginine	C01924	HMDB00670	9085
359			homocitrulline	C02427	HMDB00679	65072
360			proline	C00148	HMDB00162	145742
361			assymetric dimethylarginine (ADMA)	C03626	HMDB01539	123831
362			symmetric dimethylarginine (SDMA)	C03626	HMDB03334	169148
364			N-acetylarginine	C02562	HMDB04620	67427
365			N-acetylcitrulline	C15532	HMDB00856	656979
366			N-acetylproline			322640
367			N-delta-acetylornithine			9920500
369			N2,N5-diacetylornithine			10398396
371			trans-4-hydroxyproline	C01157	HMDB00725	5810
373			pro-hydroxy-pro		HMDB06695	11673055
376			N-methylproline			557
379			argininate*		HMDB03148	160437
382		Creatine Metabolism	guanidinoacetate	C00581	HMDB00128	763
383			creatine	C00300	HMDB00064	586
384			creatinine	C00791	HMDB00562	588
386			N-methylhydantoin	C02565	HMDB03646	69217
395		Polyamine Metabolism	acisoga			129397
396			spermine	C00750	HMDB01256	1103
399			5-methylthioadenosine (MTA)	C00170	HMDB01173	439176
400			N-acetylputrescine	C02714	HMDB02064	122356
404			4-acetamidobutanoate	C02946	HMDB03681	18189
407		Guanidino and Acetamido Metabolism	1-methylguanidine	C02294	HMDB01522	10111
408			4-guanidinobutanoate	C01035	HMDB03464	500
409			guanidinosuccinate	C03139	HMDB03157	97856
414		Glutathione Metabolism	cysteine-glutathione disulfide		HMDB00656	4247235
417			cysteinylglycine	C01419	HMDB00078	439498
419			5-oxoproline	C01879	HMDB00267	7405
422			2-hydroxybutyrate/2-hydroxyisobutyrate			
435	Peptide	Gamma-glutamyl Amino Acid	gamma-glutamylglycine		HMDB11667	165527
436			gamma-glutamylhistidine			7017195
437			gamma-glutamylisoleucine*		HMDB11170	14253342
438			gamma-glutamylleucine		HMDB11171	151023
440			gamma-glutamyl-epsilon-lysine		HMDB03869	7015685
442			gamma-glutamylphenylalanine		HMDB00594	111299
443			gamma-glutamylthreonine		HMDB29159	76078708
445			gamma-glutamyltyrosine		HMDB11741	94340
446			gamma-glutamylvaline		HMDB11172	7015683
692		Dipeptide	phenylalanylglycine		HMDB28995	98207
710			prolylglycine		HMDB11178	6426709
863		Acetylated Peptides	phenylacetylalanine			564251
865			phenylacetylhistidine			
866			phenylacetylglutamate		HMDB59772	11579826
867			phenylacetylglutamine	C04148	HMDB06344	92258
868			4-hydroxyphenylacetylglutamine			
869			phenylacetylglycine	C05598	HMDB00821	68144
873			phenylacetylmethionine			
875			phenylacetylserine			
876			phenylacetylthreonine			
877			1,5-anhydroglucitol (1,5-AG)	C07326	HMDB02712	64960
880			glucose	C00031	HMDB00122	79025

887	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	fructose 1,6-diphosphate/glucose 1,6-diphosphate/myo-inositol diphosphates	C00354		
894			3-phosphoglycerate	C00597	HMDB00807	724
896			pyruvate	C00022	HMDB00243	1060
897			lactate	C00186	HMDB00190	612
900			glycerate	C00258	HMDB00139	752
915		Pentose Metabolism	ribitol	C00474	HMDB00508	6912
916			ribonate	C01685	HMDB00867	5460677
920			xylose	C00181	HMDB00098	135191
922			arabinose	C00216	HMDB00646	66308
933			fucose	C01018	HMDB00174	19466
937			arabitol/xylitol	C01904		6912
939			arabonate/xylonate			
940			sedoheptulose		HMDB03219	5459879
953		Disaccharides and Oligosaccharides	lactose	C00243	HMDB00186	84571
963			3-sialyllactose		HMDB00825	123914
971			sucrose	C00089	HMDB00258	5988
985			maltitol/lactitol/cellobiotol/palatinol			
988		Fructose, Mannose and Galactose Metabolism	fructose	C00095	HMDB00660	5984
993			mannitol/sorbitol	C00794	HMDB00247	5780
994			mannose	C00159	HMDB00169	18950
1013			galactonate	C00880	HMDB00565	128869
1040		Aminosugar Metabolism	glucosaminate	C03752		73563
1041			glucuronate	C00191	HMDB00127	444791
1052			N-acetylneuraminate	C00270	HMDB00230	439197
1057			3'-a-sialyl-N-acetyllactosamine			
1059			6-sialyl-N-acetyllactosamine		HMDB06584	16212424
1063			N-acetylglucosaminylasparagine	C04540	HMDB00489	123826
1064			erythronate*		HMDB00613	2781043
1066			N-acetylglucosamine/N-acetylgalactosamine		HMDB00215	24139
1067		Advanced Glycation End-product	N6-carboxymethyllysine			123800
1071	Energy	TCA Cycle	citrate	C00158	HMDB00094	311
1073			aconitate [cis or trans]			
1075			isocitrate	C00311	HMDB00193	1198
1076			isocitric lactone			98259
1077			alpha-ketoglutarate	C00026	HMDB00208	51
1079			succinylcarnitine (C4-DC)		HMDB61717	71464481
1080			succinate	C00042	HMDB00254	1110
1081			fumarate	C00122	HMDB00134	444972
1082			malate	C00149	HMDB00156	525
1087			tricarballylate	C19806	HMDB31193	14925
1090			mesaconate (methylfumarate)	C01732	HMDB00749	638129
1091			citraconate/glutaconate			
1092			2-methylcitrate/homocitrate			
1096		Oxidative Phosphorylation	phosphate	C00009	HMDB01429	1061
1098		Fatty Acid Synthesis	malonylcarnitine		HMDB02095	22833583
1099			malonate	C00383	HMDB00691	867
1100			2-methylmalonylcarnitine (C4-DC)		HMDB13133	53481628
1115		Medium Chain Fatty Acid	heptanoate (7:0)	C17714	HMDB00666	8094
1252		Fatty Acid, Dicarboxylate	dimethylmalonic acid		HMDB02001	11686
1254			glutarate (C5-DC)	C00489	HMDB00661	743
1257			3-methylglutarate/2-methylglutarate		HMDB00752	
1258			2-hydroxyglutarate	C02630	HMDB00606	43
1260			adipate (C6-DC)	C06104	HMDB00448	196
1262			2-hydroxyadipate	C02360	HMDB00321	193530
1263			3-methyladipate		HMDB00555	12292
1265			maleate	C01384	HMDB00176	444266
1266			pimelate (C7-DC)	C02656	HMDB00857	385
1268			suberate (C8-DC)	C08278	HMDB00893	10457
1270			4-octenedioate		HMDB04982	11805205
1271			azelate (C9-DC)	C08261	HMDB00784	2266
1272			sebacate (C10-DC)	C08277	HMDB00792	5192

1281			3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)		HMDB61112	123979
1332		Fatty Acid Metabolism (also BCAA Metabolism)	propionylcarnitine (C3)	C03017	HMDB00824	107738
1335			methylmalonate (MMA)	C02170	HMDB00202	487
1336		Fatty Acid Metabolism (Acyl Glutamine)	hexanoylglutamine			
1337			heptanoylglutamine			
1338			N-octanoylglutamine			
1341		Fatty Acid Metabolism(Acyl Glycine)	hexanoylglycine		HMDB00701	99463
1343			3,4-methylene heptanoylglycine			
1351		Fatty Acid Metabolism(Acyl Carnitine)	acetylcarnitine (C2)	C02571	HMDB00201	1
1352			3-hydroxybutyrylcarnitine (1)		HMDB13127	53481617
1353			3-hydroxybutyrylcarnitine (2)		HMDB13127	
1372			myristoleoylcarnitine (C14:1)*			90659872
1373			suberoylcarnitine (C8-DC)			
1375			adipoylcarnitine (C6-DC)		HMDB61677	71296139
1376			3,4-methyleneheptanoylcarnitine			
1378			pimeloylcarnitine/3-methyladipoylcarnitine (C7-DC)			
1401		Carnitine Metabolism	deoxycarnitine	C01181	HMDB01161	134
1402			carnitine	C00318	HMDB00062	10917
1407		Ketone Bodies	3-hydroxybutyrate (BHBA)	C01089	HMDB00357	441
1423		Fatty Acid, Monohydroxy	2-hydroxyoctanoate		HMDB02264	94180
1435			3-hydroxypropanoate	C01013	HMDB00700	68152
1436			3-hydroxysuberate		HMDB00325	22328017
1437			3-hydroxyhexanoate			151492
1441			3-hydroxysebacate		HMDB00350	3017884
1447			5-hydroxyhexanoate		HMDB00525	170748
1451			7-hydroxyoctanoate		HMDB00486	167627
1606		Inositol Metabolism	myo-inositol	C00137	HMDB00211	892
1607			chiro-inositol	C19891	HMDB34220	
1609			scyllo-inositol	C06153	HMDB06088	892
1635		Phospholipid Metabolism	choline	C00114	HMDB00097	305
1636			choline phosphate	C00588	HMDB01565	1014
1639			glycerophosphorylcholine (GPC)	C00670	HMDB00086	71920
1641	Lipid		phosphoethanolamine	C00346	HMDB00224	1015
1643			glycerophosphoethanolamine	C01233	HMDB00114	123874
1644			glycerophosphoserine*			3081457
1645			glycerophosphoinositol*			167572
1646			trimethylamine N-oxide	C01104	HMDB00925	1145
2114		Glycolipid Metabolism	galactosylglycerol*	C05401	HMDB06790	16048618
2141		Plasmalogen	1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4)*		HMDB11352	
2176		Glycerolipid Metabolism	glycerol 3-phosphate	C00093	HMDB00126	754
2181			glycerophosphoglycerol	C03274		439964
2185		Monoacylglycerol	1-palmitoylglycerol (16:0)		HMDB31074	14900
2306		Sphingolipid Metabolism	palmitoyl sphingomyelin (d18:1/16:0)			9939941
2424		Mevalonate Metabolism	3-hydroxy-3-methylglutarate	C03761	HMDB00355	1662
2425			mevalonate	C02104	HMDB00227	439230
2426			mevalonolactone		HMDB06024	10428
2489		Pregnenolone Steroids	17alpha-hydroxypregnanolone glucuronide			
2494			21-hydroxypregnenolone disulfate			134595
2517		Progestin Steroids	pregnanediol-3-glucuronide		HMDB10318	123796
2523			pregnen-diol disulfate C21H34O8S2*			
2532			3alpha,21-dihydroxy-5beta-pregnane-11,20-dione 21-glucuronide			
2540		Corticosteroids	cortisol 21-glucuronide			
2541			cortisone	C00762	HMDB02802	222786
2551		Androgenic Steroids	11-ketoetiocholanolone sulfate			
2552			11-ketoetiocholanolone glucuronide			
2553			dehydroisoandrosterone sulfate (DHEA-S)	C04555	HMDB01032	12594
2554			16a-hydroxy DHEA 3-sulfate			
2557			epiandrosterone sulfate			9929317
2558			epiandrosterone glucuronide			10298641
2560			dehydroepiandrosterone glucuronide			
2565			androsterone sulfate		HMDB02759	159663

2568			etiocholanolone glucuronide		HMDB04484	270605
2571			androstenediol (3beta,17beta) monosulfate (1)		HMDB03818	13847309
2574			androstenediol (3beta,17beta) disulfate (1)	C04295	HMDB03818	87120982
2575			androstenediol (3beta,17beta) disulfate (2)	C04295	HMDB03818	87120982
2581			testosterone sulfate		HMDB02833	119207
2596			5alpha-androstan-3beta,17beta-diol disulfate	C12525	HMDB00493	242332
2603			andro steroid monosulfate C19H28O6S (1)*	C04555	HMDB02759	
2626		Primary Bile Acid Metabolism	cholate	C00695	HMDB00619	221493
2627			glycocholate	C01921	HMDB00138	10140
2635			tauro-beta-muricholate		HMDB00932	168408
2638			glycochenodeoxycholate glucuronide (1)			
2640			glycochenodeoxycholate sulfate			
2641			glycocholate glucuronide (1)			
2651		Secondary Bile Acid Metabolism	glycolithocholate sulfate*	C11301	HMDB02639	72222
2653			taurolithocholate 3-sulfate	C03642	HMDB02580	440071
2657			glycoursodeoxycholate		HMDB00708	12310288
2672			12-dehydrocholate		HMDB00400	94235
2674			glycocholenate sulfate*			
2675			taurocholenate sulfate			
2676			7-ketodeoxycholate		HMDB00391	188292
2679			glycodeoxycholate sulfate			
2684			ursocholate			
3250	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	inosine	C00294	HMDB00195	6021
3251			hypoxanthine	C00262	HMDB00157	790
3252			xanthine	C00385	HMDB00292	1188
3254			xanthosine	C01762	HMDB00299	64959
3257			N1-methylinosine		HMDB02721	65095
3260			urate	C00366	HMDB00289	1175
3261			allantoin	C02350	HMDB00462	204
3262			allantoic acid	C00499	HMDB01209	203
3264			1-methylhypoxanthine			70765
3270		Purine Metabolism, Adenine containing	adenosine 3',5'-cyclic monophosphate (cAMP)	C00575	HMDB00058	6076
3274			adenosine	C00212	HMDB00050	60961
3275			adenine	C00147	HMDB00034	190
3276			1-methyladenine	C02216	HMDB11599	78821
3280			N1-methyladenosine	C02494	HMDB03331	27476
3281			N6-methyladenosine		HMDB04044	1869
3290			N6-carbamoylthreonyladenosine		HMDB41623	161466
3295			2'-deoxyadenosine	C00559	HMDB00101	13730
3299			N6-succinyladenosine		HMDB00912	165243
3304		Purine Metabolism, Guanine containing	guanosine-3',5'-cyclic monophosphate (cGMP)	C00942	HMDB01314	24316
3307			guanosine	C00387	HMDB00133	6802
3308			guanine	C00242	HMDB00132	764
3310			7-methylguanine	C02242	HMDB00897	11361
3314			N1-methylguanosine		HMDB01563	96373
3315			N2-methylguanosine		HMDB05862	3035422
3316			N2,N2-dimethylguanosine		HMDB04824	92919
3317			N2,N2-dimethylguanine			74047
3326		Pyrimidine Metabolism, Orotate containing	N-carbamoylaspartate	C00438	HMDB00828	93072
3328			orotate	C00295	HMDB00226	967
3330			orotidine		HMDB00788	92751
3340		Pyrimidine Metabolism, Uracil containing	uridine	C00299	HMDB00296	6029
3341			uracil	C00106	HMDB00300	1174
3342			pseudouridine	C02067	HMDB00767	15047
3344			2'-O-methyluridine			102212
3346			N3-methyluridine		HMDB04813	99592
3352			5,6-dihydrouracil	C00429	HMDB00076	649
3357			4-ureidobutyrate			1571307
3359			3-ureidopropionate	C02642	HMDB00026	111
3360			beta-alanine	C00099	HMDB00056	239
3361			N-acetyl-beta-alanine	C01073		76406

3370		Pyrimidine Metabolism, Cytidine containing	cytidine	C00475	HMDB00089	6175
3371			cytosine	C00380	HMDB00630	597
3372			3-methylcytidine			159649
3374			N4-acetylcytidine		HMDB05923	107461
3381			2'-O-methylcytidine			150971
3391		Pyrimidine Metabolism, Thymine containing	thymine	C00178	HMDB00262	1135
3392			5,6-dihydrothymine	C00906	HMDB00079	93556
3394			3-aminoisobutyrate	C05145	HMDB03911	64956
3399	Cofactors/Vitamins	Nicotinate and Nicotinamide Metabolism	quinolinate	C03722	HMDB00232	1066
3401			nicotinate ribonucleoside	C05841	HMDB06809	161234
3403			nicotinamide	C00153	HMDB01406	936
3406			nicotinamide riboside	C03150	HMDB00855	439924
3414			nicotinamide N-oxide		HMDB02730	72661
3415			1-methylnicotinamide	C02918	HMDB00699	10129985
3420			trigonelline (N'-methylnicotinate)	C01004	HMDB00875	5570
3421			nicotinurate	C05380	HMDB03269	68499
3422			N1-Methyl-2-pyridone-5-carboxamide	C05842	HMDB04193	69698
3427		Riboflavin Metabolism	riboflavin (Vitamin B2)	C00255	HMDB00244	493570
3430		Pantothenate and CoA Metabolism	pantothenate	C00864	HMDB00210	6613
3440		Ascorbate and Aldarate Metabolism	glucarate (saccharate)	C00818	HMDB00663	33037
3441			ascorbate (Vitamin C)	C00072	HMDB00044	
3442			dehydroascorbate	C05422	HMDB01264	835
3443			threonate	C01620	HMDB00943	151152
3446			oxalate (ethanedioate)	C00209	HMDB02329	971
3447			gulonate*	C00257	HMDB03290	9794176
3449		Tocopherol Metabolism	alpha-tocopherol	C02477	HMDB01893	14985
3457			gamma-CEHC		HMDB01931	133098
3458			gamma-CEHC glucuronide*			
3459			alpha-CEHC glucuronide*			
3460			alpha-CEHC sulfate			
3470		Tetrahydrobiopterin Metabolism	biopterin	C06313	HMDB00468	445040
3471			dihydrobiopterin	C00268	HMDB00038	1879
3473		Pterin Metabolism	isoxanthopterin	C03975	HMDB00704	10729
3474			pterin	C00715	HMDB00802	73000
3475			neopterin	C05926	HMDB00845	4455
3476			7,8-dihydroneopterin	C04874	HMDB02275	65074
3478			xanthopterin			8397
3479		Hemoglobin and Porphyrin Metabolism	5-aminolevulinate	C00430	HMDB01149	137
3492			L-urobilin	C05793	HMDB04159	5280818
3498		Thiamine Metabolism	thiamin (Vitamin B1)	C00378	HMDB00235	1130
3520		Vitamin B6 Metabolism	pyridoxine (Vitamin B6)	C00314	HMDB02075	1054
3525			pyridoxal	C00250	HMDB01545	1050
3526			pyridoxate	C00847	HMDB00017	6723
3528		Benzoate Metabolism	hippurate	C01586	HMDB00714	464
3532			2-hydroxyhippurate (salicylurate)	C07588	HMDB00840	10253
3533			3-hydroxyhippurate		HMDB06116	450268
3534			4-hydroxyhippurate		HMDB13678	151012
3536			mandelate	C01984	HMDB00703	1292
3538			4-hydroxymandelate	C03198	HMDB00822	328
3540			benzoate	C00180	HMDB01870	243
3550			4-hydroxybenzoate	C00156	HMDB00500	135
3552			2,4,6-trihydroxybenzoate		HMDB29649	66520
3558			catechol sulfate		HMDB59724	3083879
3560			O-methylcatechol sulfate		HMDB60013	22473
3561			3-methyl catechol sulfate (1)			
3562			3-methyl catechol sulfate (2)			
3563			4-methylcatechol sulfate			
3571			2-ethylphenylsulfate			
3573			4-ethylphenylsulfate	C13637		
3574			4-vinylphenol sulfate	C05627	HMDB04072	6426766
3579			benzene-1,2,3-triol			

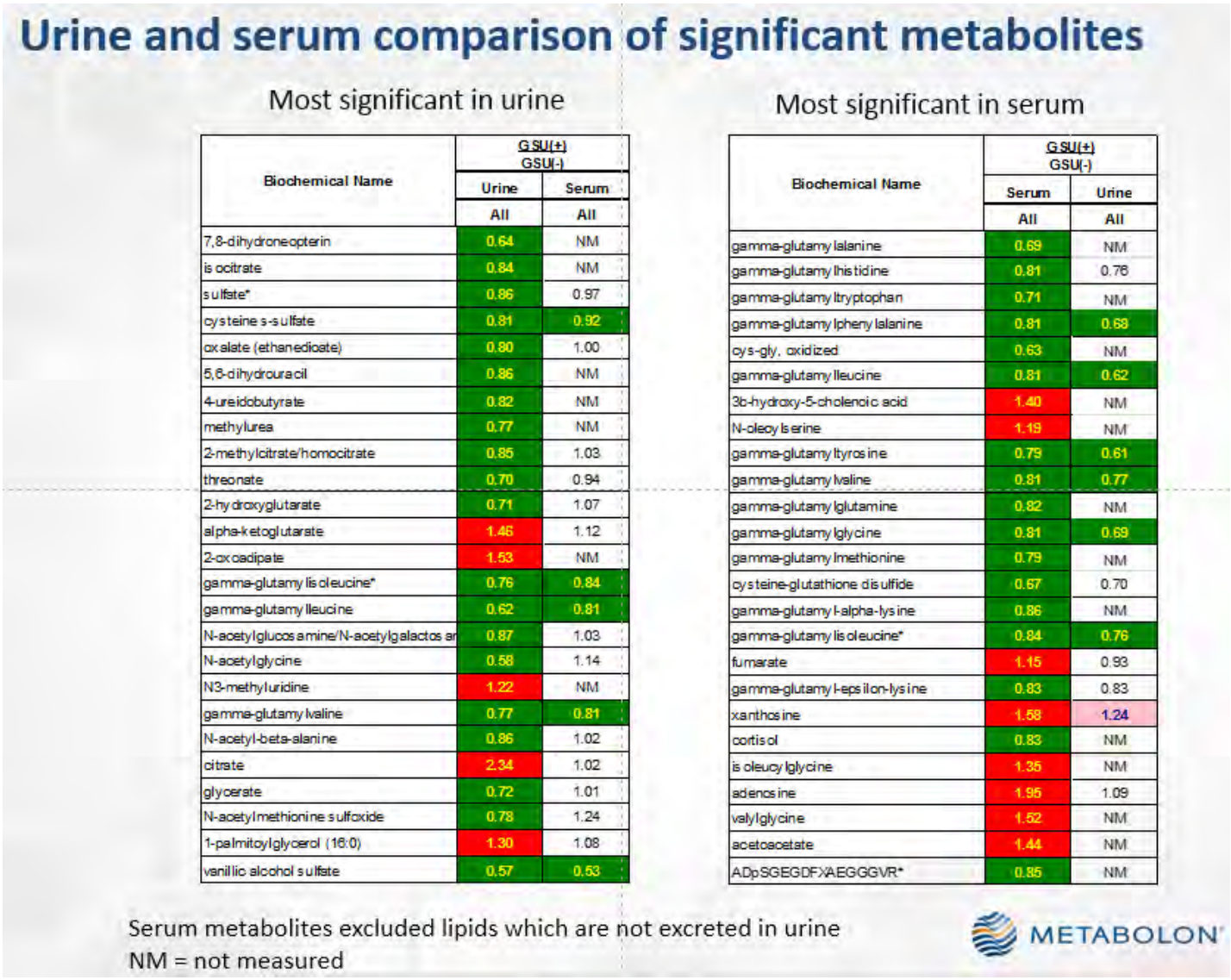
3586			3-methoxycatechol sulfate (1)			
3587			3-methoxycatechol sulfate (2)			
3588			methyl-4-hydroxybenzoate sulfate			
3590			propyl 4-hydroxybenzoate sulfate			
3595			p-cresol sulfate		HMDB11635	4615423
3602			o-cresol sulfate			11615528
3603			phenylpropionylglycine		HMDB00860	152323
3606			3-(3-hydroxyphenyl)propionate sulfate			187488
3609			3-(3-hydroxyphenyl)propionate	C11457	HMDB00375	91
3610			3-(4-hydroxyphenyl)propionate	C01744	HMDB02199	10394
3613		Xanthine Metabolism	caffeine	C07481	HMDB01847	2519
3614			paraxanthine	C13747	HMDB01860	4687
3615			theobromine	C07480	HMDB02825	5429
3616			theophylline	C07130	HMDB01889	2153
3617			1-methylurate	C16359	HMDB03099	69726
3618			7-methylurate	C16355	HMDB11107	69160
3619			1,3-dimethylurate		HMDB01857	70346
3620			1,7-dimethylurate	C16356	HMDB11103	91611
3621			3,7-dimethylurate	C16360	HMDB01982	83126
3622			1,3,7-trimethylurate	C16361	HMDB02123	79437
3623			1-methylxanthine	C16358	HMDB10738	80220
3624			3-methylxanthine	C16357	HMDB01886	70639
3625			7-methylxanthine	C16353	HMDB01991	68374
3626			5-acetylamino-6-amino-3-methyluracil	C16366	HMDB04400	88299
3627			5-acetylamino-6-formylamino-3-methyluracil	C16365	HMDB11105	108214
3629			caffeic acid sulfate		HMDB41708	
3630		Tobacco Metabolite	cotinine		HMDB01046	854019
3631			hydroxycotinine		HMDB01390	10219774
3632			cotinine N-oxide		HMDB01411	9815514
3634			3-hydroxycotinine glucuronide		HMDB01204	183115
3640			nicotine	C00745	HMDB01934	89594
3644			piperidine	C01746	HMDB34301	8082
3645			2-piperidinone		HMDB11749	12665
3683			sucralose	C12285	HMDB31554	71485
3699			1-methyl-beta-carboline-3-carboxylic acid			5406157
3717			levulinate (4-oxovalerate)		HMDB00720	11579
3720			vanillate	C06672	HMDB00484	8468
3726			1,6-anhydroglucose		HMDB00640	2724705
3728			2,3-dihydroxyisovalerate	C04039	HMDB12141	677
3729			2,3-dihydroxypyridine			28115
3732			2,8-quinolinediol sulfate			
3735			2-isopropylmalate	C02504	HMDB00402	77
3736			2-oxindole-3-acetate		HMDB35514	3080590
3743			3,5-dihydroxybenzoic acid		HMDB13677	7424
3747			3-hydroxyindolin-2-one	C11130		6097
3754			betonicine	C08269	HMDB29412	164642
3758			gluconate	C00257	HMDB00625	10690
3763			abscisate	C06082	HMDB35140	5280896
3766			alliin	C08265	HMDB33592	87310
3767			N-acetylalliin			
3777			beta-guanidinopropanoate	C03065	HMDB13222	67701
3782			3-hydroxycinnamate	C12621	HMDB01713	637541
3789			chlorogenate			5315832
3793			ciliatine (2-aminoethylphosphonate)	C03557	HMDB11747	339
3798			cinnamoylglycine		HMDB11621	709625
3805			coumaroylquinate (2)			
3807			coumaroylquinate (4)			
3808			coumaroylquinate (5)			
3810			cryptochlorogenic acid			5315599
3815			daidzein	C10208	HMDB03312	5281708
3820			dihydroferulic acid			14340

3824			enterolactone			10685477
3827			equol glucuronide			
3831			erythritol	C00503	HMDB02994	222285
3833			ferulate	C01494	HMDB00954	445858
3834			ferulic acid 4-sulfate		HMDB29200	6305574
3835		Food Component/Plant	ferulylglycine (1)			
3836			ferulylglycine (2)			
3838			fucitol			3429
3847			glucoheptose			71306729
3855			homostachydrine*	C08283	HMDB33433	441447
3859			indolin-2-one	C12312		321710
3876			maltitol		HMDB02928	3871
3881			methyl indole-3-acetate		HMDB29738	74706
3884			N-(2-furoyl)glycine		HMDB00439	21863
3888			naringenin 7-glucuronide			
3919			quinate	C00296	HMDB03072	6508
3924			saccharin	D01085	HMDB29723	5143
3926			acesulfame		HMDB33585	36573
3932			sinapate	C00482	HMDB32616	637775
3944			stachydrine	C10172	HMDB04827	115244
3945			sulforaphane		HMDB05792	5350
3947			sulforaphane-N-acetyl-cysteine			
3950			syringic acid	C10833	HMDB02085	10742
3951			tartarate	C00898	HMDB00956	444305
3952			theanine	C01047	HMDB34365	439378
3954			thymol sulfate	C09908	HMDB01878	
3966			4-allylphenol sulfate			
3975			N-acetyl-S-allyl-L-cysteine			152467
3994			4-vinylguaiacol sulfate			
3995			pyrraline		HMDB33143	122228
3996			umbelliferone sulfate			129659
3997			daidzein sulfate (2)			
3999			eugenol sulfate			180632
4001			N-acetylpyrraline			
4003			2-keto-3-deoxy-gluconate	C00204	HMDB01353	161227
4004			3-hydroxycinnamate sulfate			6443141
4005			isoeugenol sulfate			
4008	Xenobiotics		2-ketogluconate			3035456
4013			syringol sulfate			
4024			4-ethylphenol glucuronide			
4032			furaneol sulfate			
4062			Urolithin A		HMDB13695	5488186
4102		Bacterial/Fungal	N-methylpipecolate			11286529
4124			4-acetamidophenol	C06804	HMDB01859	1983
4125			3-(N-acetyl-L-cystein-S-yl) acetaminophen			83967
4126			4-acetaminophen sulfate	C06804	HMDB59911	83939
4127			4-acetamidophenylglucuronide		HMDB10316	83944
4128			2-hydroxyacetaminophen sulfate*			86290013
4129			2-methoxyacetaminophen sulfate*			86290014
4130			2-methoxyacetaminophen glucuronide*			14367271
4131			3-(cystein-S-yl)acetaminophen*			5233914
4135			2-acetamidophenol sulfate			181671
4137			4-aminophenol sulfate (2)			
4139			desmethylnaproxen		HMDB13989	13393711
4140			desmethylnaproxen sulfate			184679
4141		Drug - Analgesics, Anesthetics	ibuprofen	D00126	HMDB01925	3672
4142			2-hydroxyibuprofen		HMDB60920	10443535
4144			carboxyibuprofen		HMDB60564	10444113
4145			ibuprofen acyl glucuronide			163959
4149			salicyluric glucuronide*			
4150			acetylsalicylate		HMDB01879	2244

4162			lidocaine	D00358	HMDB14426	3676
4172			tramadol	C07153	HMDB14339	33741
4173			O-desmethyltramadol		HMDB60997	130829
4174			O-desmethyltramadol glucuronide			
4175			N-desmethyl tramadol		HMDB61007	10354700
4176			oxycodone	C08018	HMDB05024	5284603
4178			noroxycodone		HMDB41960	5489120
4187		Drug - Antibiotic	cefixime		HMDB14809	5362065
4192			cephalexin		HMDB14707	27447;6560168
4195			azithromycin	C06838	HMDB14352	2269
4199			amoxicillin	C06827	HMDB15193	2171
4200			ofloxacin	C07321	HMDB01929	4583
4201			ciprofloxacin	C05349		4011971;2764
4206			sulfamethoxazole		HMDB15150	5329
4209			doxycycline			
4227			clotrimazole	D00282	HMDB01922	2812
4232			quinine			2728270
4274		Drug - Cardiovascular	metoprolol	D02358	HMDB01932	4171
4275			metoprolol acid metabolite*			62936
4276			alpha-hydroxymetoprolol		HMDB60994	114962
4279			atenolol	D00235	HMDB01924	2249
4281			diltiazem	C06958	HMDB14487	39186
4282			verapamil	D02356	HMDB01850	2520
4292			hydrochlorothiazide	C07041	HMDB01928	3639
4293			triamterene	D00386	HMDB01940	5546
4294			furosemide	D00331	HMDB01933	3440
4295			chlorthalidone	D00272		2732
4298			valsartan			5650
4299			olmesartan	D05246	HMDB14420	158781
4300			enalapril			3222
4301			lisinopril			3937
4303			sildenafil		HMDB05039	5212
4312		Drug - Gastrointestinal	ranitidine	D00422	HMDB01930	3001055
4313			famotidine	D00318	HMDB01919	5702160
4323		Drug - Metabolic	metformin	C07151	HMDB01921	4091
4327			sitagliptin			11306691
4328			atorvastatin (lipitor)	D00887	HMDB05006	60823
4333			p-hydroxyatorvastatin			9851106
4335			rosuvastatin	D01915	HMDB15230	446157
4342			allopurinol			2094
4343			allopurinol riboside			
4344			oxypurinol	D02365	HMDB00786	4644
4357		Drug - Neurological	carbamazepine	D00252	HMDB14704	2554
4358			carbamazepine 10,11-epoxide*	C07496	HMDB60658	2555
4359			carbamazepine glucuronide*			
4362			lamotrigine	D00354		3878
4375			nicotine	C00745	HMDB01934	89594
4378			pregabalin			4715169
4382		Drug - Psychoactive	fluoxetine	D00326		3386
4384			hydroxybupropion		HMDB12235	446
4386			venlafaxine	C07187	HMDB05016	5656
4395			escitalopram	D07913	HMDB05028	146570
4412		Drug - Respiratory	diphenhydramine	C06960	HMDB01927	3100
4415			fexofenadine	C06999	HMDB05030	3348
4416			cetirizine	C07778	HMDB05032	2678
4419			dextromethorphan	C06947	HMDB01920	5362449
4423			pseudoephedrine	C02765	HMDB01943	7028
4427		Drug - Topical Agents	salicylate	C00805	HMDB01895	338
4431			hydroquinone sulfate	C00530	HMDB02434	161220
4444		Drug - Other	4-acetylphenol sulfate			4684006
4455			S-carboxymethyl-L-cysteine	C03727	HMDB29415	1080

4486			diglycerol			42953
4488			1,3-propanediol	C02457		10442
4493			sulfate*	C00059	HMDB01448	1118
4496			O-sulfo-L-tyrosine			514186
4499			2-oxo-1-pyrrolidinepropionate			3146688
4500			ethyl glucuronide		HMDB10325	152226
4511			3-acetylphenol			8487
4512			3-acetylphenol sulfate			
4514			2-aminophenol sulfate		HMDB61116	181670
4543			S-(3-hydroxypropyl)mercapturic acid (HPMA)			3371179
4558			dimethyl sulfone	C11142	HMDB04983	6213
4559			dimethyl sulfoxide (DMSO)	C11143	HMDB02151	679
4560			ectoine	C06231		126041
4565			glycolate (hydroxyacetate)	C00160	HMDB00115	757
4568			HEPES			23831
4571		Chemical	lanthionine			98504
4586			azeloylcarnitine (C9-DC)			
4587			benzoylcarnitine*			
4597			succinimide	C07273		11439
4600			triethanolamine	C06771	HMDB32538	7618
4601			trizma acetate	C07182		6503
4612			1,2,3-benzenetriol sulfate (2)			
4613			2-methoxyresorcinol sulfate			
4614			3-hydroxypyridine sulfate			
4615			1,2,3-benzenetriol sulfate (1)			
4616			3-hydroxyindolin-2-one sulfate			
4618			gentisic acid-5-glucoside			10914066
4623			4'-hydroxypropiophenone sulfate			315296
4625			6-hydroxyindole sulfate			
4628			4-acetamidobenzoate	D03836		19266
4629			thioproline			93176

Appendix 6. Comparison of statistically significant metabolites in urine and serum.



Urine and serum comparison of significant metabolites

Most significant in urine

Biochemical Name	GSU(+) GSU(-)	
	Urine	Serum
	All	All
7,8-dihydroneopterin	0.64	NM
isocitrate	0.84	NM
sulfate*	0.86	0.97
cysteine s-sulfate	0.81	0.92
oxalate (ethanedioate)	0.80	1.00
5,6-dihydrouracil	0.86	NM
4-ureidobutyrate	0.82	NM
methylurea	0.77	NM
2-methylcitrate/homocitrate	0.85	1.03
threonate	0.70	0.94
2-hydroxyglutarate	0.71	1.07
alpha-ketoglutarate	1.46	1.12
2-oxoadipate	1.53	NM
gamma-glutamylisoleucine*	0.76	0.84
gamma-glutamylleucine	0.62	0.81
N-acetylglucosamine/N-acetylgalactosa	0.87	1.03
N-acetylglycine	0.58	1.14
N3-methyluridine	1.22	NM
gamma-glutamylvaline	0.77	0.81
N-acetyl-beta-alanine	0.86	1.02
citrate	2.34	1.02
glycerate	0.72	1.01
N-acetylmethionine sulfoxide	0.78	1.24
1-palmitoylglycerol (16:0)	1.30	1.08
vanillic alcohol sulfate	0.57	0.53

Most significant in serum

Biochemical Name	GSU(+) GSU(-)	
	Serum	Urine
	All	All
gamma-glutamylalanine	0.69	NM
gamma-glutamylhistidine	0.81	0.76
gamma-glutamyltryptophan	0.71	NM
gamma-glutamylphenylalanine	0.81	0.68
cys-gly, oxidized	0.63	NM
gamma-glutamylleucine	0.81	0.62
3b-hydroxy-5-cholenoic acid	1.40	NM
N-oleoylserine	1.19	NM
gamma-glutamyltyrosine	0.79	0.61
gamma-glutamylvaline	0.81	0.77
gamma-glutamylglutamine	0.82	NM
gamma-glutamylglycine	0.81	0.69
gamma-glutamylmethionine	0.79	NM
cysteine-glutathione disulfide	0.67	0.70
gamma-glutamyl-alpha-lysine	0.86	NM
gamma-glutamylisoleucine*	0.84	0.76
fumarate	1.15	0.93
gamma-glutamyl-epsilon-lysine	0.83	0.83
xanthosine	1.58	1.24
cortisol	0.83	NM
isoleucylglycine	1.35	NM
adenosine	1.95	1.09
valylglycine	1.52	NM
acetoacetate	1.44	NM
ADpSGEGDFXAEGGGVR*	0.85	NM

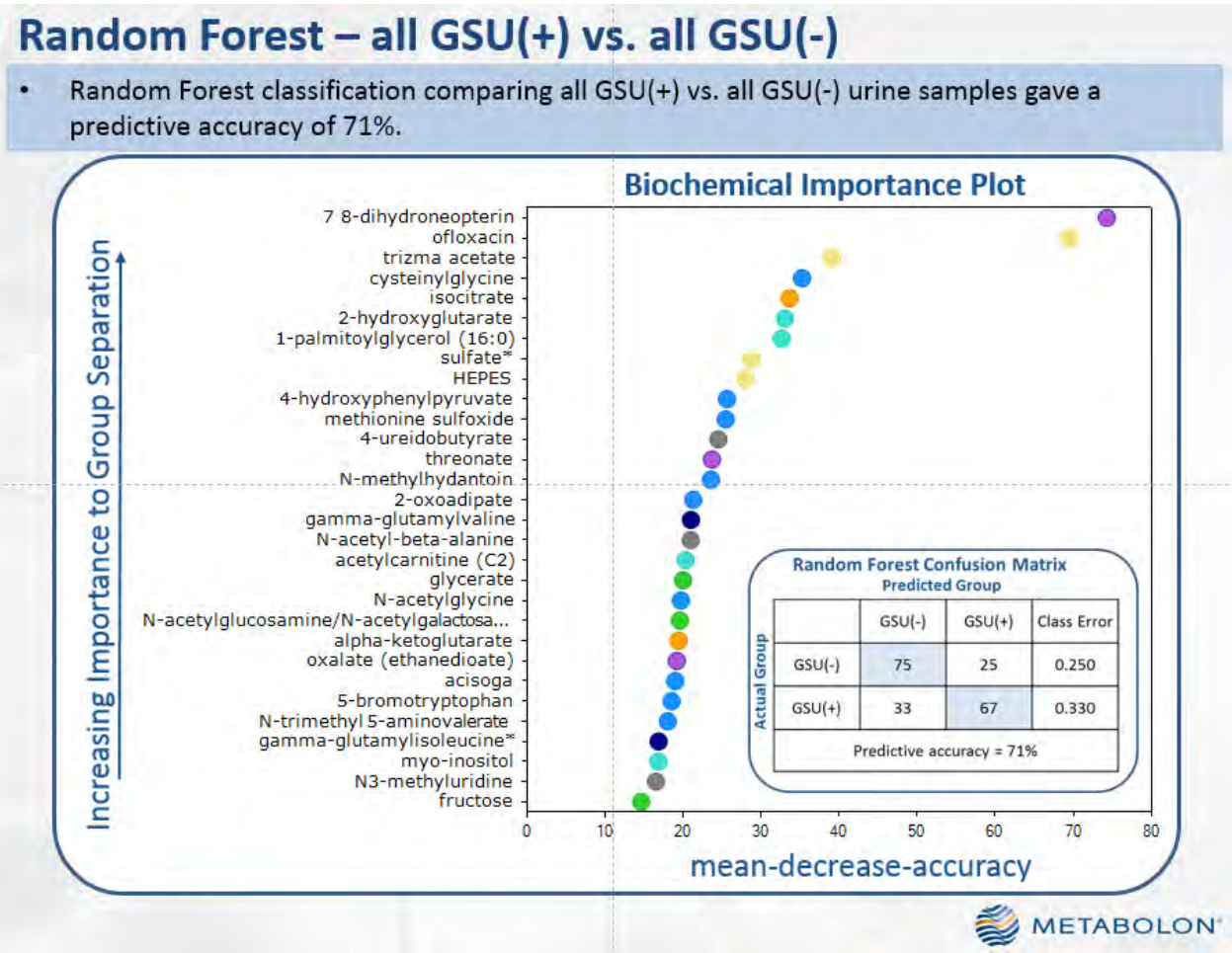
Serum metabolites excluded lipids which are not excreted in urine
NM = not measured

METABOLON

Values represent ratio of metabolite expression in cases vs. controls. Values in green are significant increased, values in red are significantly decreased. All p-values represent nominal p-values; no adjustment for multiple comparisons or false-discovery.

* Abbreviations: GSU(+), Gleason score upgrade (cases); GSU(-), Gleason score not upgraded (controls)

Appendix 7. Random forest plot of urine metabolites significantly associated with Gleason score upgrade cases (GSU+) compared to no Gleason score upgrade controls (GSU-).



* Abbreviations: GSU(+), Gleason score upgrade (cases); GSU(-), Gleason score not upgraded (controls); HEPES, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid).

Higher values of "mean-decrease-accuracy" indicate stronger associations with outcome.

Appendix 8: Meeting abstracts during reporting period: None in connection with this project

Publications during reporting period: None in connection with this project

Manuscripts in preparation:

1. Metabolomic profiling of serum to predict prostate cancer upgrading during active surveillance
2. Metabolomic profiling of urine to predict prostate cancer upgrading during active surveillance

Appendix 9: Personnel receiving pay from this negotiated effort during No Cost Extension

None